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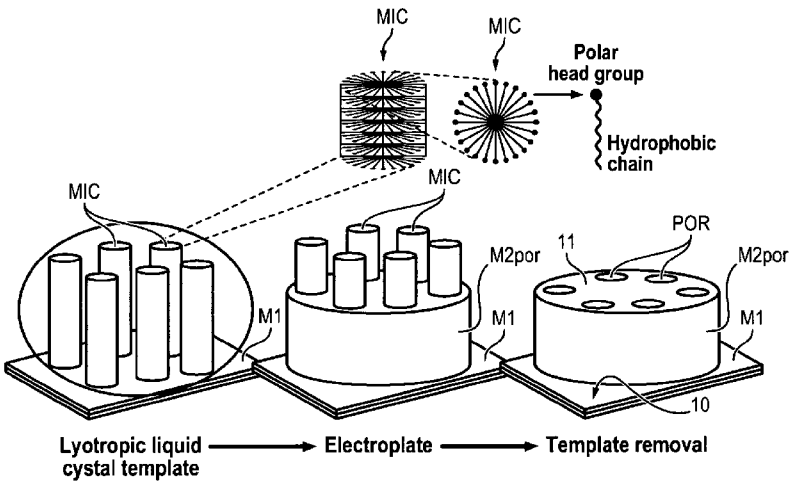
(54) **Title**: DEVICE TO STIMULATE OR RECORD A SIGNAL TO OR FROM A LIVING TISSUE

FIG. 12a




(57) **Abstract**: The invention concerns a device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode comprising a zone for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone. According to the invention, the zone (11) for application to the living tissue belonging to the first electrode (10) and/or supplementary conductive surface (3) has a first geometric surface made of at least one first conducting material and modified by an external organized mesoporous layer consisting in at least one second conducting material (M2por) having pore (POR) diameters within 1-10 nm.

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**Device to stimulate or record a signal to or from a living tissue**

The invention concerns a device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode and/or supplementary conductive surface comprising a zone for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone, wherein the zone for application to the living tissue is connected to said conductor.

The present invention solves issues encountered when recording or stimulating neural networks using metal electrodes, and especially using microelectrodes.

A current challenge is to build efficient microelectrode arrays (MEAs) for recording and electrically stimulating electrogenic cells (neurons, glial cells, muscular cells, cardiac cells, stem cells,). The present invention is illustrated for the case of neurons but equally applies to any electrogenic cells other than neurons.

The stake of neural stimulation device is to build neural prosthesis or implants to compensate function loss due to a lesion or the degeneration of a part of the Central Nervous System (CNS). For this purpose, two main approaches are considered: macrostimulation using macroscopic electrodes or macroelectrodes (surface of several $mm^2$), or microstimulation using microelectrodes (below several hundreds of $(\mu m)^2$). Macroscopic stimulation of the brain is used in routine to suppress tremors in patients suffering from Parkinson diseases, while macrostimulation of the spinal cord alleviates chronic neuropathic pain. More recently, microstimulation has gained increasing interest to achieve more focal and specialized stimulations of restricted groups of neurons. This is of particular interest for the development of sensory neural prosthesis such as cochlear, retinal, subcortical, or cortical implants.

As described in patent application WO 2009/053333, the focality of an electrical stimulation can be improved by introducing a supplementary conductive surface (also called ground surface when connected to the ground) surrounding the

electrodes of the array. The focality is all the higher that the surface conductance of the supplementary conductive surface is high.

In order to achieve high surface conductance, microelectrodes coated with black platinum have been developed. However, black platinum is fragile, making these electrodes not stable along time and in addition not very well defined in terms of surface roughness and/or porosity.

One problem of those devices applied to a living tissue is that they should be able to record signals of very low amplitude from, and/or send sufficiently high electric currents due to the application to a living tissue. It is wished to send sufficiently high signals to the living tissue or to receive very small signals from the living tissue.

Thus, recording noise and current injection capabilities should be improved.

The goal of the invention is to have a well defined device enabling to send sufficiently high or receive very small electric signals to or from a living tissue.

According to the invention, the surface of the zone for application to the living tissue is modified by an external porous layer.

A subject matter of the invention is a device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode and/or supplementary conductive surface comprising a zone for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone, wherein the zone for application to the living tissue is connected to said conductor,

characterized in that

the zone for application to the living tissue belonging to the first electrode and/or supplementary conductive surface has a first geometric surface made of at least one first conducting material and modified by an external mesoporous layer consisting in at least one second conducting material having pore diameters within 1-10 nm .

According to an embodiment of the invention, the zone for application to the living tissue has an active surface of contact for application to the living tissue, with the modification of the first geometric surface by the external mesoporous layer

being such that the active surface of the application zone is increased by at least an order of magnitude, without changing the geometric surface, wherein n orders of magnitude is equal to $10^n$.

According to an embodiment of the invention, the external mesoporous layer has a developed surface area increased by at least an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

According to an embodiment of the invention, the zone for application to the living tissue has an active surface of contact for application to the living tissue, with the modification of the first geometric surface by the external mesoporous layer being such that the active surface of the application zone is increased by at least 2 orders of magnitude, without changing the geometric surface, wherein n orders of magnitude is equal to $10^n$.

According to an embodiment of the invention, the external mesoporous layer has a developed surface area increased by at least 2 orders of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

According to an embodiment of the invention, the zone for application to the living tissue has an active surface of contact for application to the living tissue, with the modification of the first geometric surface by the external mesoporous layer being such that the active surface of the application zone is increased by more than an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

According to an embodiment of the invention, the external mesoporous layer has a developed surface area increased by more than an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

According to an embodiment of the invention, the external mesoporous layer has a developed surface area, which is determined based on a cyclic voltamogram measured on the first electrode before and after said modification of the first geometric surface by the external mesoporous layer.

According to an embodiment of the invention, the developed surface area is increased from the geometric surface by a factor equal to

$(ADSA)_{after\text{-}mod} / (ADSA)_{before\text{-}mod}$

wherein

$(ADSA)_{before\text{-}mod}$ is the area of a prescribed abyss (A) calculated on the voltamogram measured on the first electrode before said modification of the first geometric surface by the external mesoporous layer,

$(ADSA)_{after\text{-}mod}$ is the area of the prescribed abyss (A) calculated on the voltamogram measured on the first electrode after said modification of the first geometric surface by the external mesoporous layer,

the external mesoporous layer has a developed surface area DSA which is proportional to the area $(ADSA)_{after\text{-}mod}$ measured on the voltamogram of the electrode.

According to an embodiment of the invention, the developed surface area DSA of the external mesoporous layer is equal to

$$DSA = GS \,.\, (ADSA)_{after\text{-}mod} / (ADSA)_{before\text{-}mod} ,$$

wherein

GS is the geometric surface area.

According to an embodiment of the invention, the zone for application has a width D

$1\ \mu m \leq D \leq 100\ \mu m$.

According to an embodiment of the invention, $4\ \mu m \leq D \leq 20\ \mu m$.

According to an embodiment of the invention, $8\ \mu m \leq D \leq 16\ \mu m$.

According to an embodiment of the invention, $10\ \mu m \leq D \leq 14\ \mu m$.

According to an embodiment of the invention, the zone (11) for application has a width of 12 μm.

According to an embodiment of the invention, the external mesoporous layer consisting in said at least one second material being porous of the first geometric surface of the first electrode is formed on a first underlying material of the zone for application to the living tissue connected to said conductor.

According to an embodiment of the invention, the geometric area of the first geometric surface of the zone for local application to the living tissue is greater than $0.1\ (\mu m)^2$ and smaller than $400\ (\mu m)^2$.

According to an embodiment of the invention, the geometric area of the first geometric surface of the zone for local application to the living tissue is greater than 50000 $(\mu m)^2$ and smaller than 10 $cm^2$.

According to an embodiment of the invention, the geometric area of the first geometric surface of the zone for local application of the supplementary conductive surface to the living tissue is greater than 100 $(\mu m)^2$ and smaller than 1000 $cm^2$.

According to an embodiment of the invention, the mesoporous layer has a thickness between 1 nm and 10 μm.

According to an embodiment of the invention, the porous layer has pores arranged in a spatially organized way.

According to an embodiment of the invention, the porous layer has a uniform pore size obtained with micelles MIC of identical sizes.

According to another embodiment of the invention, the porous layer has different pore sizes obtained using a mixture of micelles MIC of different sizes. For instance, the porous layer may have pore sizes following a Gaussian distribution defined by a mean value and a standard deviation.

According to an embodiment of the invention, the external porous layer consisting in said at least one second material being porous is formed on the first underlying material of the zone for application to the living tissue connected to said conductor, wherein the at least one first material is a conductive material which is the same as the at least one second material.

According to an embodiment of the invention, the external porous layer consisting in said at least one second material being porous is formed on the first underlying material of the zone for application to the living tissue connected to said conductor, wherein the at least one first material is a conductive material which is different from the at least one second material.

According to an embodiment of the invention, the external porous layer is made of Pt, Au, Ir, TiN, Ir oxyde, Tungstene, ITO, semiconductors, alloys, conducting polymers or any other conducting material.

According to an embodiment of the invention, the first electrode is a first microelectrode and an array of first microelectrodes is provided, wherein the array

of first microelectrodes is arranged in a determined configuration side–by–side, wherein each first microelectrode can be selected, the first microelectrodes being insulated from each other, the conductors being insulated from each other, the zones for local application to the living tissue being insulated from each other.

According to an embodiment of the invention, a plurality of third conductive zones are located, respectively for a determined plurality of first microelectrodes, in the vicinity of the plurality of zones of said determined first microelectrodes of the array and are insulated from these zones for application to the living tissue, said plurality of conductive third zones belongs to a third conductive surface supplemental to the first microelectrodes and separate from the first microelectrodes, the third supplemental conductive surface being used for all or part of the application against the living tissue, connection means being provided to ensure electric connection between said plurality of third conductive zones of the supplemental third surface for all said determined plurality of first microelectrodes insulated from one another, so that the third conductive zones of the third supplemental surface are substantially equipotential, the supplemental third conductive surface also being connected to at least one port intended to be connected to an external return conductor returning at least part of the signal and being formed to ensure focal stimulation from at least one of the determined plurality of first microelectrodes and to act as common focusing means for several zones of different determined first microelectrodes.

According to an embodiment of the invention, the second mesoporous layer is fabricated by electrodeposition of the second conducting material through a template defining the pores, said template having surfactant that is washed out after the electrodeposition.

According to an embodiment of the invention, the second mesoporous layer is fabricated by electrodeposition of the second conducting material through a template defining the pores, said template being composed of surfactant that is washed out after the electrodeposition.

According to an embodiment of the invention, the pores are made using micelles made of the surfactant and having a defined size and a defined concentration

in the second conducting material, wherein the micelles are washed out leaving in the second material the pores of size and concentration corresponding to the defined size and defined concentration of the micelles.

According to an embodiment of the invention, the micelles are in such a concentration and such a uniform size so as to touch one another so as to leave at least some contiguous pores among said pores.

According to an embodiment of the invention, for said mesoporous modification, a charge density of the second material lower than or equal 8 $C/cm^2$ is electrodeposited on the first geometric surface of the zone for application.

According to an embodiment of the invention, the charge density of the second material has a value not exceeding 2 $C/cm^2$.

According to an embodiment of the invention, the charge density of the second material has a value not exceeding 1 $C/cm^2$.

Another subject matter of the invention is a device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode and/or supplementary conductive surface comprising a zone for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone, wherein the zone for application to the living tissue is connected to said conductor,

characterized in that

the zone for application to the living tissue belonging to the first electrode and/or supplementary conductive surface has a first geometric surface made of at least one first conducting material and modified by an external macroporous layer consisting in at least one second conducting material.

According to an embodiment of the invention, the macroporous layer has pore diameters within 50-1000 nm.

According to an embodiment of the invention, the macroporous layer has pore diameters of 500 nm.

According to an embodiment of the invention, the surface of the zone for application to the living tissue has an external porous modified layer to have an

intrinsic recording voltage noise at least 10% smaller than that achieved with the same but not porous material.

According to an embodiment of the invention, the zone for application to the living tissue belonging to the first electrode and/or supplementary conductive surface has a first geometric surface formed of an external porous layer and preferentially organized porous layer consisting in at least one second material being porous and arranged in such a manner that the first electrode has a standard deviation of intrinsic recording voltage noise $N_{M2por}$ measured under defined conditions according to the following first formula

$$N_{M2por} \leq 0.9\, N_{M2}\ ,$$

wherein $N_{M2}$ is the standard deviation of the intrinsic recording voltage noise measured under the same defined conditions with another second comparison electrode and/or supplementary conductive surface being the same as the first electrode having its zone for application to the living tissue having the same first geometric surface but formed of said at least one second material (M2) being not porous and especially not forming an organized porous layer,

wherein said conditions comprise measuring the voltage in a bandwith between 1 and 3000 Hz in a physiological liquid at 25 °C.

The inventors have discovered that well defined macroporous or mesoporous modification of electrodes lead to a reduction of noise level by at least 10% with respect to the same flat material, or to a reduction of noise level according to the first formula. The second comparison electrode does not belong to the device according to the invention.

According to an embodiment, $N_{M2por} \leq 0.75\, N_{M2}$.

According to another embodiment, $N_{M2por} \leq 0.5\, N_{M2}$.

According to an embodiment of the invention, according to the second following formula :

$$\log (N_{M2por}) \leq 1.5 - 0.57 \log (D),$$

wherein

log is the decimal logarithm,

D is a width of the zone of the first electrode in μm,

N is in μV.

In an embodiment, the physiological liquid is a Ringer's solution.

In an embodiment, the physiological liquid is composed of (in mM): 113 NaCl, 4.5 KCl, 2 $CaCl_2 2H_2O$, 1 $MgCl_2 6H_2O$, 25 $NaHCO_3$, 1 $NaH_2PO_4 H_2O$ and 11 D-Glucose.

Noise reduction depends on the width or diameter of the microelectrode.

One critical parameter of electrodes used to record or stimulate the CNS is the surface of contact with the extracellular conductive medium (active surface). A large active surface leads to better performances for recording and stimulation:

Regarding recording, a low intrinsic noise level of the electrode is obtained.

Regarding electrical stimulation, the capacity to inject higher currents through the electrode without damage of the electrode and the tissue due to electrochemical reaction at the interface.

In an embodiment,

$\log (N_{M2por}) \leq 1.5 - 0.6 \log (D)$,

wherein

log is the decimal logarithm,

D is a width of the zone of the first electrode in μm,

N is in μV.

In an embodiment, the standard deviation of intrinsic recording voltage noise $N_{M2por}$ of the first electrode is lower than 3 μV for the zone (11) for local application to the living tissue having a width D between 12 μm and 64 μm.

The porosity of the layer(s) is organized and controlled, for example by the fact that the pores have uniform size. The arrangement of the pores is controlled. The number of pores is proportional to the injected charge density used for the modification by the second material. The injected charge density is a parameter used to control the porosity. Of course several porous layers can be superimposed on the first geometric surface.

In an embodiment, for the porous layer(s) in general, which can be for example mesoporous or macroporous, for said porous modification, the porosity is controlled by a charge density of the second material, lower than or equal 8 $C/cm^2$

electrodeposited on the first geometric surface of the zone (11) for application, and which can have a value not exceeding 2 C/cm$^2$, for example not exceeding 1 C/cm$^2$.

For example the pores are in contact with each other.

According to an embodiment of the invention, the impedance of each microelectrode is ≤ 500 kΩ and preferentially ≤ 120 kΩ.

According to an embodiment of the invention, the zone (11) for application to the living tissue belonging to the first electrode (10) and/or supplementary conductive surface (3) has a first geometric surface made of at least one first conducting material and modified by an external organized mesoporous or macroporous layer consisting in at least one second conducting material (M2por).

The invention will be better understood on reading the following description given solely as a non–limiting example with reference to the appended drawings in which:

- Figures 1A, 1B, 1C, 1D, 1E, 1F and 1G are schematic view of examples of configurations of a set of electrodes according to the invention.

- Figure 1H is a schematic view of a set of microelectrodes surrounded by a supplementary conductive surface;

- Figure 2A is a microscopic photo of an example of a macroporous planar disk microelectrode;

- Figure 2B is a microscopic photo of a macroporous 3D conical microelectrode (B);

- Figure 3 is a microscopic photo of an example of a mesoporous planar disk microelectrode;

- Figure 4 shows a typical cyclic voltamogram of a gold microelectrode before (interrupted line) and after macroporous modification (continuous line);

- Figure 5 shows a typical cyclic voltamogram of a platinum microelectrode before (interrupted line) and after mesoporous modification (continuous line);

- Figures 6A and 6B show an example of noise reduction obtained with a macroporous microelectrode before and after macroporous modification;

- Figures 7A and 7B show an example of noise reduction following mesoporous modification;

- Figure 8 shows noise reduction obtained with mesoporous modification for 5 microelectrodes of different diameters;

- Figure 9 shows example of action potentials recorded using mesoporous microelectrodes;

- Figure 10 shows an example of a local field potential recorded using mesoporous microelectrodes,

- Figure 11a shows three steps of fabrication of microelectrodes on a substrate,

- Figure 11b shows an overall perspective view of a device manufactured according to an embodiment of the invention,

- Figure 11c shows a view of microlectrodes according to an embodiment of the invention,

- Figure 11d shows an example of noise recording for different electrode sizes manufactured according to an embodiment of the invention,

- Figure 11d and 11e respectively show the noise level $\sigma_e$ and the impedance (Figure 11f) measured on microlectrodes according to an embodiment of the invention having different microelectrode diameters, while Figure 11g shows the noise level $\sigma_e$ as a function of the impedance of the same,

- Figure 12a shows steps of fabrication of microelectrodes having a mesoporous layer according to an embodiment of the invention,

- Figure 12b shows the tip of an example of a flat microelectrode and figure 12c shows the tip a mesoporous microelectrode manufactured according to an embodiment of invention,

- Figure 13a shows an example of a cyclic voltammogram (Intensity of current in the microelectrode measured as a function of measured voltage of the microelectrode) for a microelectrode according to an embodiment of the invention,

- Figure 13b shows the reduction peak charge determined based on measured voltamograms of microelectrodes manufactured according to an embodiment of the invention for different microelectrode diameters,

- Figure 14a illustrates the frequency-dependent impedance amplitude of microelectrodes according to an embodiment of the invention for different amounts of charge used for their nanostructuration and for flat (non porous) microelectrodes,

- Figure 14b shows the impedance of microelectrodes according to an embodiment of the invention for different microelectrode diameters,

- Figure 15a shows the voltage measured on a microelectrode before (on the left) and after (on the right) modification according to an embodiment of the invention,

- Figure 15b shows the intrinsic noise level $\sigma_e$ as a function of the charge density injected for porous modification for different microelectrode diameters according to an embodiment of the invention,

- Figure 15c shows both the impedance and intrinsic noise level $\sigma_e$ which have been measured for microelectrodes of an array having different sizes according to an embodiment of the invention and for flat electrodes,

- Figure 15d shows the intrinsic noise levels $\sigma_e$ which have been measured for several microelectrodes of an array according to an embodiment of the invention and for flat electrodes before (flat) and after porous modification,

- Figure 15e shows the mean noise improvement due to modification of Figure 15d, whereas Figure 15f shows the value of the intrinsic noise level $\sigma_e$ measured before modification divided by the intrinsic noise level $\sigma_e$ measured after modification according to Figure 15e,

- Figure 16a shows an embryonic mouse hindbrain-spinal cord preparation on an array of microelectrodes according to an embodiment of the invention shown by the points,

- Figure 16b shows voltage signal recordings of signals received from an embryonic mouse hindbrain-spinal cord preparation using an array of microelectrodes according to an embodiment of the invention shown by the points of Figure 16a distributed on said preparation,

- Figure 16c shows a close-up view of two successive episodes recorded on 2 electrodes of the array of Figure 16b,

- Figure 16d shows the same data segment of Figure 16c after high-pass filtering,

- Figure 16e shows the same recording as Figure 16d using flat microelectrodes.

In the drawings, each first electrode 10 is electrically-conductive and has a zone 11 for application to a living tissue, which is connected to a conductor for sending a signal voltage to the living tissue in order to stimulate the living tissue or for receiving a signal voltage from the living tissue in order to record a signal from the living tissue. The zone 11 is for example situated at the end of the conductor. The device can comprise a single electrode 10 or a plurality of electrodes 10. The electrode 10 can be a microelectrode 10, for example as in figures 1A, 1B, 1C, 1D, 1E and 1F or a macroelectrode 10 for example as in figure 1G, or a supplementary conductive surface as in Figure 1H.

For example, the device to stimulate a living tissue comprises an array 1 of electrodes 10 arranged in a determined configuration side–by–side, each electrode 10 comprising a zone 11 for local application to the living tissue and a conductor for sending a stimulation signal, wherein each electrode 10 can be selected for application of an electric stimulation signal or the reception of a signal from the living tissue, the electrodes 10 being insulated from each other, the conductors being insulated from each other, the zones 11 being insulated from each other, as in examples of Figures 1A, 1B, 1C, 1D, 1E and 1F.

In an embodiment, the present invention consists in using 2D or 3D microelectrodes with a small geometric surface (typically for example 1 to 10000 $(\mu m)^2$) but showing a high active macroporous or mesoporous surface to record or stimulate electrogenic cells. This is further illustrated in the case of neurons, but can equally apply to any other electrogenic cells, such as glial cells, muscular cells, cardiac cells, or stem cells. Moreover the present invention is illustrated in the case of microelectrodes for which it is expected to bring important benefits. However, the invention also applies to macroelectrodes. Also, the present invention is illustrated for microelectrode arrays obtained by microfabrication (e.g., lithography) but can equally apply to isolated microwires or arrays of microwires, the non-isolated tips of

which being used as a microelectrode, as in example of Figure 1E. Different examples of electrodes and arrays of electrodes for which the invention applies are illustrated in Figures 1A, 1B, 1C, 1D, E , 1F and 1G. Of course the invention applies also to a single electrode or a set of electrodes or an array of electrodes with regular or irregular spacing and even positions in different planes in 3D.

In the embodiment of Figure 1A, each microelectrode 10 of an array 1 of microelectrodes has a zone 11 for application to a living tissue, which has a disk-shaped planar external surface and which is connected to a conductor for sending a signal voltage to the living tissue in order to stimulate the living tissue or for receiving a signal voltage from the living tissue in order to record a signal from the living tissue. For example, the zone 11 is transverse to the direction L of application against the living tissue. Of course, the zone 11 can have a planar or not planar (3D) external surface not being disk-shaped.

In Figure 1H, a supplementary third conductive surface 3 for application also against the living tissue is provided in the vicinity of the zone(s) 11. The supplementary conductive surface 3 is isolated from the zones 11. The supplementary conductive surface 3 is for example in Figures 1H in the form of a grid. The supplementary conductive surface 3 can be also for example in the form of a plan or any other shape surrounding the electrodes. The supplementary conductive surface 3 comprise a plurality of conductive third zones 13 being located, respectively for a determined plurality of electrodes 10, in the vicinity of the plurality of first zones 11 of said determined electrodes 10 of the array and are insulated from these zones 10. Said plurality of conductive zones 13 belongs to the conductive surface 3 supplemental to the electrodes 10 and separate from the electrodes 10, the supplemental conductive surface 3 being used for all or part of the application against the living tissue. Connection means are provided to ensure electric connection between said plurality of conductive zones 13 of the supplemental surface 3 for all said determined plurality of electrodes 10 insulated from one another, so that the conductive zones 13 of the supplemental surface 3 are substantially equipotential. The supplemental conductive surface 3 is also connected to at least one port intended to be connected to an external return conductor of the

signal and being formed to ensure focal stimulation from at least one of the determined plurality of electrodes 10 and to act as common focusing means for several application zones 11 of different determined electrodes 10. The surface 3 is described in document WO 2009/053333 which is incorporated herein by reference.

In the embodiment of Figure 1B, each zone 11 for application to a living tissue has an external surface in the form of a tip, which is for example conical (shown by a triangle on Figure 1B).

In the embodiment of Figure 1C, a plurality of zones 11 are disposed along the application length L of an insulating substrate 4, wherein the length L is directed towards the living tissue and the substrate has a form narrowing towards the living tissue, for example in the form of a tip. Depending of how deep each substrate 4 will penetrate the living tissue, a different number of zones 11 will be in contact with the living tissue. A multiplicity of substrates 4 carrying each one or a plurality of zones 11 can be provided. Since the substrate can be provided along two first and second dimensions and the zones 11 in the third dimension L perpendicular to the two first and second dimensions, a three dimensional array of electrodes is provided. In figure 1C, one first dimension is provided to form a linear shank of substrates 4. In Figure 1D, the two first and second dimensions are provided.

In the embodiment of Figure 1E, each zone 11 for application to a living tissue has an external surface which is formed by the end of a wire 12, wherein the wire 12 serves as conductor for sending a signal voltage to the living tissue in order to stimulate the living tissue or for receiving a signal voltage from the living tissue in order to record a signal from the living tissue.

In the embodiment of Figure 1F, each zone 11 for application to a living tissue has an external surface which is cylindrical along an insulating tube 5, wherein the zones 11 form part of the external surface of the tube 5 and are disposed one behind the other along the tube 5.

In the embodiment of Figure 1G, the zone 11 for application is of a single macroelectrode and is for example disk-shaped, for example for EEG scalp recording and is connected to said conductor 12.

According to the invention, the zone 11 for application to the living tissue belonging to the first electrode 10 has a first geometric surface formed of an external porous layer consisting in at least one second material M2por being porous and arranged in such a manner that the first electrode has a standard deviation of intrinsic recording voltage noise $N_{M2por}$ measured under defined conditions according to the following first formula

$$N_{M2por} \leq 0.9\, N_{M2} ,$$

wherein $N_{M2}$ is the standard deviation of the intrinsic recording voltage noise measured under the same defined conditions with another second comparison electrode being the same as the first electrode 10 but having its zone 11 for application to the living tissue having the same first geometric surface formed of said at least one second material M2 being non porous.

For example, the external porous layer consisting in said at least one second material M2por being porous of the first geometric surface of the first electrode 10 is formed on a first underlying material M1 of the zone 11 for application to the living tissue, wherein the first underlying material M1 is connected to said conductor.

Porous zones 11 of metal electrodes 10 or of supplementary conductive surface 3 consisting in M2por are obtained for example by controlled modification of their surface so as to increase the active surface (developed surface) of their application zone 11 by several orders of magnitude without noticeably changing their geometrical surface. This is achieved by electrodeposition of a metal or metals, semiconductors, alloys, conducting polymers or other conducting materials M2por through a template that will define the porosity of the resulting surface.

After electrodeposition, the underlying material M1 may possibly be removed leaving only the porous layer of M2por forming the zone 11 for application to the living tissue. The deposited surface M2por may have a typical thickness of several tens or hundreds of nanometers to a few microns. The geometric surface of the zone 11 is the surface on which the porous external layer is formed and is called geometric, since its geometric area can be calculated from the overall dimensions of the zone 11. Of course, other intermediate layers including mesoporous or macroporous layers can be added between the geometric surface and said external

porous layer. For example, in case of figures 1A, 1C, 1D, 1E and 1G, the width D is the diameter of the zone 11 having the first geometric surface being circular, the geometric surface of zone 11 is a disk having a known predetermined diameter D and a corresponding geometric surface area equal to $\pi.D^2/4$. In case of figure 1B, the geometric surface of zone 11 is a cone of known predetermined angle. In case of figure 1F, the geometric surface of zone 11 is a cylinder of known dimensions. The geometric surface of the zone 11 is called first geometric surface and can have one or several first underlying material(s) M1.

The external porous layer on the first geometric surface of the zone 11 for application to the living tissue is formed of one or several second porous material(s) M2por, which can be the same as the first material(s) M1 or can be different from the first material(s) M1. Consequently in the electrode according to the invention, called first electrode, the second porous material(s) M2por is on the first material(s) M1.

The fabrication of the porous layer M2por uses salts or molecules as precursors for the electroformation of the final porous conducting structure. Therefore the toxicity is highly dependent on the type of precursor employed. For example, salts based on platinum and gold which are both noble metals and therefore not toxic themselves are used. The surfactants that are employed to generate the porosity, like octaethyleneglycol or similar molecules, do not present a significant toxicity because in practice the surfactant is washed out after deposition.

In an embodiment, the zone 11 for application to the living tissue is metallic or an alloy or semiconductor or polymer or any other conducting material and the zone 11 for application to the living tissue is made of an underlying material M1 being metallic or an alloy or semiconductor or polymer or any other conducting material, with the second porous external layer made of a material M2por being also metallic or alloy or semiconductor or polymer or any other conducting material on the zone 11.

In an embodiment, the second porous external layer is of the same or different metallic material or materials M2por as the underlying material M1 of the zone 11 for application to the living tissue.

In an example, the second porous external layer and the first underlying material of the first geometric surface of zone 11 for application to the living tissue are made of platinum, i.e.:

M1 = Pt and M2por = Pt.

In another example, the second porous external layer and the first underlying material of the first geometric surface of zone 11 for application to the living tissue are made of gold, i.e.:

M1 = Au and M2por = Au.

In the frame of the current invention, two types of porous structures of M2por are considered:

Macroporous electrodes obtained by electrodeposition of a metal, semiconductor, alloy, conducting polymer or other conducting materials through a colloidal crystal, typically made of latex or silica beads.

Mesoporous electrodes obtained by electrodeposition of a metal, semiconductor, alloy, conducting polymer or other conducting materials from a mixture as described in previous patent US6503382.

In an embodiment, a mesoporous layer can be formed onto a macroporous layer.

The obtained porous layer generally has a uniform pore size, but may have different pore sizes obtained using a mixture of micelles MIC (mesoporous case) or beads (macroporous case) of different sizes. For instance, the porous layer may have pore sizes following a Gaussian distribution defined by a mean value and a standard deviation.

The obtained porous first microelectrodes are very stable over time, unlike platinum black electrodes which are not considered organized porous but rough and are very fragile and friable and do not last a long time.

The following results were achieved:

Fabrication of macroporous and microporous first microelectrodes

Arrays of gold or platinum first microelectrodes were modified using macroporous, and mesoporous approaches, respectively. Macroporous electrodes

had pore diameters of about 500 nm (Figures 2A and 2B), while mesoporous microelectrodes had pore diameters within 1-10 nm (Figure 3 for the example of a 32 μm diameter mesoporous microlelectrode).

In the example of macroporous electrodes, spheres of 500 nm diameter were used to make the pores.

In examples of mesoporous electrodes, the pores were made using micelles of 1.75 nm +/-0.2 nm diameter.

Examples of noise reduction using mesoporous modification are given in Figure 8 showing examples of noise level reduction in ordinate using mesoporous modification for different sizes of zone 11 of the first microelectrode between 28 and 3200 $(\mu m)^2$ in abscissa, with a zone 11 being a plane disk of diameter D according to Figure 1A. The first underlying material of the geometric surface of electrode and zone 11 are made of platinum with a porous layer of platinum, i.e. M1 = Pt , M2por =Pt and M2 = Pt in the example of Figure 8. In this case, the geometric surface of zone 11 for application against the living tissue is a disk of diameter D, whose geometric area S is equal to $\pi.D^2/4$. The noise level of the instrumentation serving to measure the noise was $\sigma_a$ = 1 μV. Consequently, on Figure 8, the intrinsic RMS voltage noise level of the electrode having zone 11 for application against the living tissue has to be corrected.

Here are for different values of the diameter D of zone 11 for application against the living tissue:

- the measured standard deviation N2MES of the recording voltage noise level, indicated by a disk on Figure 8 for the first porous electrode having the first geometric surface of zone 11 of diameter D for application against the living tissue, wherein the first geometric surface of said first porous electrode is formed by the second external porous layer made of the second porous material M2por (N2MES = $\sigma_s$),

- the corrected standard deviation $N_{M2por}$ of the recording voltage noise level, which is obtained by a correction of the measured standard deviation N2MES of the recording voltage noise level, and which is equal to the standard deviation of intrinsic recording voltage noise $N_{M2por}$ measured for the first porous electrode

having the first geometric surface of zone 11 of diameter D for application against the living tissue, wherein the first geometric surface of said first porous electrode is formed by the second external porous layer made of the second porous material M2por ($N_{M2por} = \sigma_e$), :

$N_{M2por} = ((N2MES)^2 - 1)^{1/2}$ for $\sigma_a = 1$ µV).

- the measured standard deviation N3MES of the recording voltage noise level N3MES indicated by a square on Figure 8 for said second non porous comparison electrode, which is another electrode which has been fabricated with the same first geometric surface of zone 11 of diameter D for application against the living tissue, wherein said other second comparison electrode is the same as the first electrode 10 but has not the geometric surface of its zone for application to the living tissue formed by the porous layer consisting in the second porous material M2por, wherein the first geometric surface of the zone for application to the living tissue of said other second comparison electrode is formed of said at least one second material M2 being non porous;

- the corrected standard deviation $N_{M2}$ of the recording voltage noise level N3MES for the second non porous comparison electrode, which is obtained by a correction of the measured standard deviation N3MES of the recording voltage noise level N3MES, and which is equal to the standard deviation of the intrinsic recording voltage noise $N_{M2}$ for the second non porous comparison electrode, which is said other electrode having the same first geometric surface of zone 11 of diameter D for application against the living tissue, wherein said other second comparison electrode is the same as the first electrode 10 but has not the geometric surface of its zone for application to the living tissue formed by the porous layer consisting in the second porous material M2por, wherein the first geometric surface of the zone for application to the living tissue of said other second comparison electrode is formed of said at least one second material M2 being non porous :

$N_{M2} = ((N3MES)^2 - 1)^{1/2}$

| Diameter D (µm ) | N3MES (non porous), µV | Intrinsic $N_{M2}$ (non porous), µV | N2MES (porous), µV | Intrinsic $N_{M2por}$ (porous), µV |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 6 | 14.13 | 14.09 | 6.39 | 6.31 |
| 12 | 8.40 | 8.34 | 2.99 | 2.82 |
| 16 | 7.19 | 7.12 | 2.76 | 2.57 |
| 32 | 5.22 | 5.12 | 2.25 | 2.02 |
| 64 | 3.32 | 3.16 | 1.78 | 1.47 |

In a general manner, for a first geometric surface of zone 11 of any prescribed shape, the value D is called the width of the zone 11 for application against the living tissue and is calculated by the following second formula

$$D = (4.S/\pi)^{1/2}$$

wherein S is the area of the geometric surface of the zone 11,

wherein the second porous layer is situated on said first geometric surface of the zone 11. Then, the zone 11 can have any geometric surface, for example rectangular or square or any shape in a plane, but also non plane shapes, like for example conic or others.

When the first geometric surface of zone 11 has the second porous external layer, the second porous external layer has a developed (active) surface area DSA which is proportional to an area ADSA measured on the voltamogram of the electrode, as described below. The developed surface area DSA of the second porous external layer of the first electrode is higher than the area of its first geometric surface.

Electrochemical characterization of macroporous and mesoporous first electrodes:

Microelectrodes were characterized using cyclic voltametry before and after macroporous or mesoporous modifications. In the example of figure 4, macroporous modification of gold microelectrodes led to an increase of the active surface by a factor of 6, with M1 =Au, M2por = Au and M2 = Au. In the example of figure 5, mesoporous modification of a platinum microelectrode with M1 =Pt, M2por =Pt and M2 = Pt leads to an increase of the active surface by more than an order of magnitude, wherein n orders of magnitude are equal to $10^n$.

Figure 4 shows a cyclic voltamogram (CV) of the current in ordinate depending on voltage E in abscissa for a gold microelectrode, before the porous

modification (interrupted line, second electrode) and after the macroporous modification (continuous line, first electrode).

Figure 5 shows a cyclic voltamogram (CV) of the current in ordinate depending on voltage E in abscissa for a platinum microelectrode, before the porous modification (interrupted line, second electrode) and after the mesoporous modification (continuous line, first electrode).

The voltamogram of the porous electrode has an abyss A whose area ADSA on the voltamogram is proportional to the developed surface area DSA of zone 11. Said abyss A reaches much higher absolute values than the corresponding voltamogram for the non porous electrode of same width D. The developed surface area DSA of zone 11 is determined from the voltamogram and enables to verify the porosity of the porous layer, in order to have the required noise level for width D.

A method to constitute the first electrode can be the following.

In order to have a prescribed noise N3MES, the corresponding width D is determined on figure 8 according to equation

Log (N3MES) = 1.6 – 0.6 log (D),

to obtain the width D of the non porous second electrode.

An equivalent non porous geometric surface area S of the zone 11 for application to a living tissue is determined by

$S = \pi.D^2/4$.

Then the value of the developed surface area DSA of the porous layer that should be brought on the first geometric surface for the first electrode is rendered equal to S

DSA = S.

For another method, DSA > S.

Then the voltamogram of the first electrode, on the zone 11 of which the porous layer has been brought, is determined to have an area ADSA of abyss A corresponding to the calculated value of the developed surface area DSA to determine the features to impose to the porous layer based on ADSA.

Noise reduction for neural signal recording of the first electrode:

Macroporous modification leads to a reduction of noise level by a factor up to about 1.5 for a 40-µm gold microelectrode (Figures 6A and 6B).

Mesoporous modification leads to a stronger reduction of noise by factors of typically x3 for a 12-µm diameter planar platinum electrode, as shown in Figures 7A and 7B.

Figure 6A shows the signal voltage in ordinate depending on time in abscissa before the porous modification (second electrode) and figure 6B shows the signal voltage in ordinate depending on time in abscissa after the porous modification for a macroporous modification (first electrode).

Figure 7A shows the signal voltage in ordinate depending on time in abscissa before the porous modification (second electrode) and figure 7B shows the signal voltage in ordinate depending on time in abscissa after the porous modification (first electrode) for a mesoporous modification of a 12-µm-diameter microelectrode.

Porous electrode modification yields 2 advantages for neural stimulation. First, the increased active surface of first microelectrodes allows the injection of higher currents while remaining in the reversible domain and thus without electrode and tissue damage. In a subsidiary manner, the increased active surface of the supplementary third conductive surface 3 yields a more focal stimulation.

Hereunder are described examples of fabrication of electrodes according to the invention.

Fabrication of MEAs with different electrode sizes

In order to highlight the influence of electrode diameter on intrinsic noise level, and further assess the benefit of mesoporous modifications, 56-channel microelectrode array MEA with different electrode sizes (4, 6, 8, 12, 16, 32, 64 µm) have been developed (Figure 11a, 11b, 11c), with electrodes arranged in a 4x15 layout without corners covering an area of 900x12600 $\mu m^2$ adapted to the geometry of embryonic hindbrain-spinal cord preparations, or a 1x56 linear arrangement with an inter-electrode spacing of 150 µm. The different electrode sizes were achieved

with a metal layer of unique size (80 μm) covered by a silicon nitride insulation layer having an opening of variable size. These arrays were compatible with MultichannelSystems amplifier MEA1060 in order to be tested for neural recording. Figure 11a illustrates the fabrication process. First, an initial insulating substrate 100 consisting in for example either glass or silicon wafer covered by a 500-nm oxide layer is patterned with a metal layer (here Platinum) defining the electrodes 10 and their leads by a lift-off technique. For this purpose, the wafer is spin-coated with 2 μm of NLOF 2020 (Clariant) photoresist, which is further removed at specific locations by photolithography to define the geometry of the metal layer. Next, 100 nm of titanium and 150 nm of platinum are deposited over the whole wafer. The wafer is then placed in a dedicated remover AZ400 to remove the photoresist, leaving only the metal layer deposited directly on the initial substrate at the location of the electrodes and leads. In a second step, an insulation layer 200 of silicon nitride is deposited by PECVD (Plasma Enhanced Chemical Vapor Deposition) over the whole wafer. This silicon nitride layer is then etched at the location of the electrodes. For this purpose, the wafer is protected by a NLOF photoresist film (2 μm), which is etched over the electrodes with openings of different sizes to define the different electrode zones 11 for application. Then, the silicon nitride is etched using a plasma of SF6 gas at locations not protected by the photoresist (i.e., electrodes). NLOF is then removed. Finally, the wafer is cleaned and cut to separate the MEAs and an annular glass ring was glued with PDMS to make a recording chamber around the electrodes. Figure 11b shows an MEA obtained on a glass substrate. Figure 11c shows an example of resulting electrodes of different sizes on a silicon substrate. Of course, the invention can be embodied on electrodes of different sizes and also on electrodes having the same size.

Mesoporous modification of microelectrodes:

Figure 12a outlines the key steps that were necessary to fabricate mesoporous microelectrodes on a microelectrode array. A nanostructured metal film on the microelectrodes having first material M1 was obtained by electroplating the second metal material M2POR in the presence of lyotropic liquid crystalline phases,

acting as a template. Here, platinum ions that were dissolved in the aqueous domains of the liquid crystalline phases were electrochemically reduced. This resulted in a platinum deposit around the surfactant molecules that were arranged in a rod like configuration as micelles MIC. Washing away the surfactant, i.e washing away the micelles MIC, after the electroplating revealed an array of mesopores POR in the metal deposit M2.

The plating mixture consisted of the ternary system composed of 42wt% octaethylene glycol monododecyl ether ($C_{12}EO_8$, 98% purity, Sigma Aldrich), 29wt% hexachloroplatinic acid hydrate ($H_2PtCl_6$ , 99.9% purity, Sigma Aldrich), and 29wt% Milli-Q reagent water (resistivity $\geq$ 18MΩ). The components were mixed in a glass vial vigorously for several minutes at room temperature until a gel-like compound was obtained. The closed vial was then placed in a thermostated oven at ~40°C for 30 min to allow the mixture to homogenize. Mixing and the subsequent heating steps were repeated until a homogeneous sample was obtained.

The viscous, orange-colored plating mixture was applied simultaneously to all the microelectrodes on the microelectrode array. An Ag/AgCl electrode and a platinum wire (diameter 1mm) were put into the plating mixture, to serve as reference and counter electrode respectively. The microelectrodes of the microelectrode array were the working electrodes. To reduce the platinum ions present in the plating mixture the potential was stepped from a value of +0,6V to -0,1V until the desired amount of charge has passed. In order to study the influence of the film thickness on the physical properties of the electrode, different amounts of charge ranging from 1 to 8 C/cm$^2$ were applied to single microelectrodes on the microelectrode array. In some experiments electrodeposition was done simultaneously on all microelectrodes until a fixed value of charge was passed. After the electrodeposition step the plating mixture was removed and the microelectrodes were rinsed with copious amounts of water to wash away the surfactant. To make sure that no surfactant remains in the pores POR, a piranha solution (attention: dangerous solution), composed of 75vol% $H_2SO_4$ (95-98% $H_2SO_4$, Merck) and 25vol% hydrogen peroxide ($H_2O_2$, 30% solution, Fluka), was allowed to react with the microelectrodes for 10 minutes followed by a washing procedure with distilled

water. Scanning electron micrographs of the electrodes were recorded by a Hitachi Tabletop Microscope TM-1000, using an accelerating voltage of 15 kV. Figure 12b shows an example of a flat microelectrode of 8-µm diameter and figure 12c shows a mesoporous microelectrode of 8-µm diameter.

The concentration and size of the micelles MIC enables to control the density and size of the pores POR. In an embodiment, at least some of the pores are contiguous. In this case, the micelles MIC touch one another, i.e. are contiguous, so as to leave at least some contiguous pores POR among said pores. For example, the micelles MIC have a uniform size to have pores POR of uniform size.

Electrochemical characterization

The electrochemical characterization of microelectrodes on the MEA was performed using cyclic voltammetry (CV) before and after electrodeposition in order to compare the active surface area of electrode. Cyclic voltammograms were recorded with a scan rate of $100mV.s^{-1}$ in 0.5 M sulfuric acid (SDS) that was bubbled with nitrogen for 5 min prior to the experiment. All electrochemical experiments were performed using a µAutolab type III potentiostat (Eco Chemie) with a conventional three-electrode cell configuration, with an Ag/AgCl (sat. KCl) electrode as reference and a platinum wire as counter electrode. The reduction peak charge Q is calculated from the voltamogram as ADSA / V, wherein V is the scanning speed in Volt/second.

Impedance measurements

Impedance measurements were performed in the same physiological liquid as that used for neural tissue recoding (in mM: 113 NaCl, 4.5 KCl, 2 $CaCl_2 2H_2O$, 1 $MgCl_2 6H_2O$, 25 $NaHCO_3$, 1 $NaH_2PO_4 H_2O$ and 11 D-Glucose) using an Autolab PGSTAT 12 (EcoChemie, Metrohm) potentiostat equipped with a frequency response analyzer (FRA module). Measurements were recorded between 10 kHz and 1 Hz with an AC amplitude of 10 mV peak to peak and 6 points per decade of frequencies. The applied working potential during the measurement was maintained at 0.3V vs. Ag/AgCl.

Intrinsic electrode noise measurements

To measure the intrinsic noise level of the electrodes, the electrical potential was recorded for 1 minute in physiological liquid between each of the 60 microelectrodes and an Ag/AgCl ground electrode pellet. Signals were 1100× amplified and band-pass filtered between 1 Hz and 3 kHz using MCS MEA1060-Up-BC filter amplifiers from Multi Channel Systems (Reutlingen, Germany). Data were acquired at 10 kHz using two synchronized CED Power1401 AD converter and the Spike2 v6 software from Cambridge Electronic Design (Cambridge, England). The standard deviation of the signal $\sigma_s$ was then calculated over the 1-min recording for each electrode of the array. Because this noise level was composed of both the intrinsic noise level of the electrodes $\sigma_e$ and the electronic noise level of the amplifiers $\sigma_a$, it was assumed statistical independence of these two noise and estimated the intrinsic noise level $\sigma_e$ of each electrode as:

$$\sigma_e = \sqrt{\sigma_s^2 - \sigma_a^2}$$

were $\sigma_a$ was measured with the amplifier inputs connected to the ground.

Neural recordings

Low-noise neural recordings were obtained from whole embryonic mouse hindbrain-spinal cord preparations. In brief, E13.5 embryos were surgically removed from pregnant OF1 mice (Charles River Laboratories, L'Arbresle, France) previously killed by cervical dislocation. The whole spinal cord and medulla were dissected in the physiological liquid gassed with carbogen (95% $O_2$, 5% $CO_2$), meninges were removed, and the neural tube was opened along the rostro-caudal axis (open-book preparation) and then placed over the microelectrodes in the MEA chamber and superfused with physiological liquid gassed with carbogen. A plastic net with small holes (70×70 $\mu m^2$) was laid on the neural tissue, in order to achieve a tight and uniform contact with the microelectrodes. Spontaneous rhythmic activity of this immature preparation was recorded for several hours at room temperature using the same apparatus has for the noise measurements.

Noise level and impedance depends on electrode diameter

It was first assessed how the noise level and impedance of the microelectrodes depended on their diameter for the MEAs. Figure 11d shows an example of noise recording for different electrode sizes. It can be seen that the smaller the electrode, the higher the noise, with peak-to-peak amplitudes of typical measurements in the range of 40-60 µV for electrode diameters below 16 µm. As further quantified for all the electrodes of an array, the noise level (Figure 11e) and the impedance (Figure 11f) were inversely proportional to the electrode diameter, and thus linearly correlated (Figure 11g). Figure 11e shows that in this example

$\sigma_e = 1.58+63.14 / D$ ,

wherein D is the electrode diameter (diameter of zone 11).

Mesoporous microelectrodes have high active surfaces

Figure 12b and 12c show an SEM image of a flat and a mesoporous 8-µm diameter microelectrode, respectively. The size of the mesopores lies around 2nm and is defined by the lyotropic liquid crystal template used during the electrodeposition. This pore size is too small to be resolved in SEM images, but nevertheless surface morphology of the electrodeposited film could be assessed at a lower resolution. Figure 12c reveals a homogenous, very smooth platinum film. A slightly thicker deposit of platinum can be observed at the perimeter of the electrode originating from a more efficient radial diffusion occurring on microelectrodes. However for thin deposits, not exceeding an injected charge density of 2 $C/cm^2$, this phenomenon was not observed.

In order to compare flat with mesoporous microelectrodes, cyclic voltammetry experiments were performed before and after electrodeposition. Figure 13a shows an example of cyclic voltammograms for 12-µm diameter platinum microelectrodes having or not a nanostructured surface. In one oxidation-reduction cycle, the current observed for the mesoporous electrode is increased by more than one order of magnitude compared to the flat microelectrode. This increase is

attributed to the well-ordered, highly accessible array of mesopores on the electrode surface.

To quantitatively assess the gain in active surface area, a measure of the area ADSA of the reduction peak A of Platinum in the CV (dashed area in Figure 13a, obtained by integration over the corresponding reduction peak) was carried out. This area represents the total charge associated to the reduction of Platinum during one cycle and is thus proportional to the active surface of the electrode. Figure 13b shows this total charge as a function of the electrode diameter for unmodified and nanostructured electrodes, giving $(ADSA)_{after\text{-}mod}$ represented by full squares and $(ADSA)_{before\text{-}mod}$ represented by empty squares. Electrodeposition of a mesoporous overlayer onto microelectrodes resulted roughly in a 100-fold enhancement of their active surface area for all the diameters tested (from 8 to 64 microns).

Mesoporous microelectrodes of low impedance

Impedance spectroscopy was performed to determine the reduction of impedance obtained by mesoporous modification (within the range 1-1000 Hz). Figure 14a illustrates the frequency-dependent impedance amplitude of 12-µm diameter microelectrodes for different amount of charge used for their nanostructuration. In theory, the higher the charge, the thicker the nanostructured layer, and thus the lower the impedance. Nanostructuration reduced impedances by about 1 or 2 orders of magnitude, the highest reduction being obtained for lowest frequencies. Increasing the amount of charge used for the modification above 1 $C/cm^2$ improved electrode impedance only moderately. The impedance reduction for different electrode diameters was evaluated in Figure 14b each for 1 Hz, 10 Hz, 100 Hz and 1000 Hz. As shown in Figure 14b, a reduction by x5-10 was generally obtained at all frequencies for all three diameters tested (6, 8, 12 µm), systematically with strongest improvements for 12 µm microelectrodes (> x10). A flat electrode is the electrode before the porous modification, i. e. having the same geometric surface without the mesoporous layer of second conducting material.

According to an embodiment of the invention, the impedance of each microelectrode with a geometrical surface of 113 $(\mu m)^2$ (12-µm diameter) is $\leq$ 500 kΩ and preferentially $\leq$ 120 kΩ.

Mesoporous microelectrodes of low intrinsic noise

The improvement brought by the mesoporous structuration of the electrode surface was evaluated in terms of intrinsic recording noise level. Figure 15a shows an example of noise reduction obtained for a 12-µm-diameter microelectrode. When testing different modification charges, no significant improvement was obtained by increasing the amount of charge beyond 1 $C/cm^2$ (Figure 15b). Figure 15c shows both the impedance and intrinsic noise level of flat microelectrodes of an array having different sizes (square symbols) showing a linear correlation as in Figure 11g. In this graph, 12-µm electrodes are represented by open squares and their noise and impedance obtained after nanomodification are shown by open circles symbols (same electrodes). It can be seen that these small-size microelectrodes present the even better features than 32- and 64-µm flat electrodes. Finally, the reproducibility of the noise improvement was tested on an array composed of 56 12-µm microelectrodes for which electrodes were modified with a charge of 1 $C/cm^2$. As shown in Figure 15d, the noise improvement was homogenous across the array, with noise decreasing from 8.4 ± 1.1 µV (mean ± SD) to 3.3 ± 1.2 µV (Figure 15e), corresponding to an average reduction by a factor of 2.72 ± 0.63 (Figure 15f).

Low-noise neural recordings with small size mesoporous microelectrodes

Rhythmic activity was recorded in whole embryonic mouse hindbrain-spinal cord preparations (Figure 16a) using MEAs made of 12-µm-diameter mesoporous platinum microelectrodes. Activity was composed of rostro-caudal waves originating in the hindbrain and propagating caudally along the spinal cord (Figure 16b). Figure 16c shows a close-up view of two successive episodes recorded on 2 electrodes of the array. Each episode is composed of a local-field potential superimposed on a burst of spikes. Figure 16d shows the same data segment after high-pass filtering, on which low-amplitude bursts of spikes can easily be seen. As illustrated in Figure 16e,

these bursts could not have been clearly seen using conventional 12-μm flat platinum microelectrodes.

In Figure 14b the loss in deposition efficency expected for the smaller microelectrodes is compensated by higher charge densities that were passed to these during electrodeposition.

In the example of the microelectrode fabricated as indicated above in reference to figures 11a to 16e of the invention, having a zone 11 for application of diameter D = 12 micrometers and modified by the second mesoporous layer :

M1 = Pt,

M2por = Pt,

$(ADSA)_{after\text{-}mod}$ = 5.59 nC,

$(ADSA)_{before\text{-}mod}$ = 18.9 pC,

Geometric surface = 113.1 $(\mu m)^2$,

DSA (after modification by the mesoporous layer) = 33450 $(\mu m)^2$,

DSA (before modification by the mesoporous layer) = 113.1 $(\mu m)^2$,

Active surface of contact (after modification by the mesoporous layer) = DSA (after modification by the mesoporous layer),

Active surface of contact (before modification by the mesoporous layer) = DSA (before modification by the mesoporous layer),

Pore size = 17.5 Angstrom,

Size of the micelles = 17.5 Angstrom,

Concentration of the micelles = 42 wt % (weight percentage),

Uniform pore size = yes,

Micelles of above-mentioned surfactant having concentration = $10^{12}$ to $10^{13}$ $cm^{-2}$,

Micelles of above-mentioned surfactant having a uniform size = yes,

Charge density of the second material = 2 $C/cm^2$,

Impedance = 103 kΩ at 1000 Hz,

N2MES = $\sigma_e$ = 2.40 μV,

$N_{M2por}$ = $\sigma_e$ = 1.93 μV,

N3MES = $\sigma_e$ = 6.48 μV,

$N_{M2}$ = $\sigma_e$ = 6.33 μV,

Thickness of mesoporous layer = from 50 to 800 nm.

The present invention allows solving the following problems for recording or stimulating electrogenic cells:

Reduction of noise level using microelectrodes or macroelectrodes for recording electrical signals originating from electrogenic cells either in vitro or in vivo, e.g.: action potentials, local field potentials, scalp electroencephalography (EEG), electrocorticograms (ECoG) using epi- or sub-dural electrodes, stereotaxic EEG (SEEG) using implanted macroscopic electrodes;

Higher current injection capability using microelectrodes or macroelectrodes either in vitro or in vivo;

Higher stimulation focality of electrical stimulation using the approach of a supplementary second conductive surface surrounding the electrodes of an array;

Increasing active surface of microelectrodes for electrogenic cell recording and stimulation;

Increasing active surface of macroelectrodes for electrogenic cell recording and stimulation;

Increasing active surface of the supplementary conductive section for increased focality of stimulations.

The field of application of the invention comprises :

Exploration of neural networks in vitro or in vivo using microelectrodes or macroelectrodes and arrays of such, like microelectrode arrays (MEAs), EEG, SEEG, or ECoG;

Development of neural implants for functional rehabilitation (e.g., cochlear, retinal, spinal cord, cortical, sub-cortical, hippocampic implants);

Exploration of other types of electrogenic cells, such as cardiac cells, muscles, vessel cells, stem cells, pancreatic cells;

Development of cardiac implants for recording of physiological signals or stimulation of the heart, part of the heart, or part of the cardio-vascular system.

In an embodiment, the geometrical surface of zones 11 of first microelectrodes is greater than 0.1 $(\mu m)^2$ and smaller than 100 $(\mu m)^2$.

In an embodiment, the geometrical surface of zones 11 of first microelectrodes is greater than 0.1 $(\mu m)^2$ and smaller than 400 $(\mu m)^2$.

In an embodiment, the first microelectrodes 10 may be bi-dimensional or three-dimensional.

In an embodiment, the first microelectrodes 10 may be microwire sections or parts of a microwire's surface.

In an embodiment, the geometrical surface of first macroelectrodes 10 is greater than 50000 $(\mu m)^2$ and smaller than 10 $cm^2$.

In an embodiment, the thickness of the porous layer is between 1 nm and 10 µm.

Electrode materials M1 can be: Pt, Au, Ir, Ir oxyde, TiN, Tungstene, ITO, semiconductors, alloys, conducting polymers or any other conducting material.

A modified first microelectrode array (MEA) was used for the recording of the activity of an embryonic mouse hindbrain and spinal cord in order to show its application for real measurements. Immediately after dissection of the mouse embryo, the neural tissue was put onto the electrode sites of the MEA that was filled with Ringer solution. In this structure, two types of neural signals were recorded using mesoporous platinum microelectrodes of 30 µm diameter: action potentials (Figure 9 showing voltage in ordinate depending on time in abscissa) and local field potential (Figure 10 showing voltage in ordinate depending on time in abscissa with measurement of local field potential LFP).

The improved electro-chemical properties of the modified array contribute to lower noise content compared to the non-modified one and thus the recordings of extracellular signals can benefit from the apparent improvement of signal-to-noise ratio (SNR), since the quality of the recorded extracellular signals can be evaluated

in terms of their SNR characteristics, the overall signal amplitude and the shape of the signal. The better recording performances of the modified electrodes allow to see cellular events that otherwise would have been hidden by the noise and we can therefore conclude on an overall increase in the performance of the recording or stimulating system.

Below are shown example of RMS noise level values before (second electrode) and after mesoporous modification (first electrode) for three electrodes of different diameter 2 μm, 32 μm and 64 μm:

| Diameter (μm) - | RMS before (μV) - | RMS after (μV) - | Factor of reduction |
|---|---|---|---|
| 2 | 17.83 | 4.71 | 3.8 |
| 32 | 6.73 | 2.22 | 3 |
| 64 | 4.36 | 2.79 | 1.6 |

From the resulting RMS values one can see that the highest noise reduction factor was obtained for the electrode with the smallest diameter (2 μm) and the lowest noise reduction was obtained for the biggest electrode (64μm).

The noise measurements of the figures were performed in a Ringer solution composed of (in mM): 113 NaCl, 4.5 KCl, 2 $CaCl_2 2H_2O$, 1 $MgCl_2 6H_2O$, 25 $NaHCO_3$, 1 $NaH_2PO_4 H_2O$ and 11 D-Glucose.

# CLAIMS

1. Device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode (10) and/or supplementary conductive surface (3) comprising a zone (11) for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone (11), wherein the zone (11) for application to the living tissue is connected to said conductor,

characterized in that

the zone (11) for application to the living tissue belonging to the first electrode (10) and/or supplementary conductive surface (3) has a first geometric surface made of at least one first conducting material and modified by an external mesoporous layer consisting in at least one second conducting material (M2por) having pore diameters within 1-10 nm .

2. Device according to claim 1, characterized in that the zone (11) for application to the living tissue has an active surface of contact for application to the living tissue, with the modification of the first geometric surface by the external mesoporous layer being such that the active surface of the application zone (11) is increased by at least an order of magnitude, without changing the geometric surface, wherein n orders of magnitude is equal to $10^n$.

3. Device according to any one of the preceding claims, characterized in that the external mesoporous layer has a developed surface area (DSA) increased by at least an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

4. Device according to claim 1, characterized in that the zone (11) for application to the living tissue has an active surface of contact for application to the living tissue, with the modification of the first geometric surface by the external mesoporous layer being such that the active surface of the application zone (11) is increased by more than an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

5. Device according to any one of the claims 1 and 4, characterized in that the external mesoporous layer has a developed surface area (DSA) increased by more than an order of magnitude, without changing the geometric surface, wherein n orders of magnitude are equal to $10^n$.

6. Device according to any one of the claims 1 to 5, characterized in that the external mesoporous layer has a developed surface area (DSA), which is determined based on a cyclic voltamogram (CV) measured on the first electrode before and after said modification of the first geometric surface by the external mesoporous layer.

7. Device according to claim 6, characterized in that the developed surface area (DSA) is increased from the geometric surface by a factor equal to

$(ADSA)_{after\text{-}mod} / (ADSA)_{before\text{-}mod}$

wherein

$(ADSA)_{before\text{-}mod}$ is the area of a prescribed abyss (A) calculated on the voltamogram measured on the first electrode before said modification of the first geometric surface by the external mesoporous layer,

$(ADSA)_{after\text{-}mod}$ is the area of the prescribed abyss (A) calculated on the voltamogram measured on the first electrode after said modification of the first geometric surface by the external mesoporous layer,

the external mesoporous layer has a developed surface area DSA which is proportional to the area $(ADSA)_{after\text{-}mod}$ measured on the voltamogram of the electrode.

8. Device according to claim 7, characterized in that the developed surface area DSA of the external mesoporous layer is equal to

$$DSA = GS \,.\, (ADSA)_{after\text{-}mod} / (ADSA)_{before\text{-}mod},$$

wherein

GS is the geometric surface area.

9. Device according to any one of the preceding claims, characterized in that the zone (11) for application has a width D

$1\ \mu m \leq D \leq 100\ \mu m$.

10. Device according to claim 9, characterized in that $4\ \mu m \leq D \leq 20\ \mu m$.

11. Device according to claim 9, characterized in that $8\ \mu m \leq D \leq 16\ \mu m$.

12. Device according to claim 9, characterized in that 10 μm ≤ D ≤ 14 μm.

13. Device according to claim 9, characterized in that the zone (11) for application has a width of 12 μm.

14. Device according to any one of the preceding claims, characterized in that the external mesoporous layer consisting in said at least one second material (M2por) being porous of the first geometric surface of the first electrode (10) is formed on a first underlying material (M1) of the zone (11) for application to the living tissue connected to said conductor.

15. Device according to any one of the preceding claims, characterized in that the geometric area of the first geometric surface of the zone (11) for local application to the living tissue is greater than 0.1 $(\mu m)^2$ and smaller than 400 $(\mu m)^2$.

16. Device according to any one of the preceding claims, characterized in that the geometric area of the first geometric surface of the zone (11) for local application to the living tissue is greater than 50000 $(\mu m)^2$ and smaller than 10 $cm^2$.

17. Device according to any one of the preceding claims, characterized in that the geometric area of the first geometric surface of the zone (11) for local application of the supplementary conductive surface (3) to the living tissue is greater than 100 $(\mu m)^2$ and smaller than 1000 $cm^2$.

18. Device according to any one of the preceding claims, characterized in that the mesoporous layer has a thickness between 1 nm and 10 μm.

19. Device according to any one of the preceding claims, characterized in that the porous layer has a uniform pore size.

20. Device according to any one of claims 1 to 19, characterized in that the external porous layer consisting in said at least one second material (M2por) being porous is formed on the first underlying material (M1) of the zone (11) for application to the living tissue connected to said conductor, wherein the at least one first material (M1) is a conductive material which is the same as the at least one second material (M2).

21. Device according to any one of the claims 1 to 19, characterized in that the external porous layer consisting in said at least one second material (M2por) being porous is formed on the first underlying material (M1) of the zone (11) for

application to the living tissue connected to said conductor, wherein the at least one first material (M1) is a conductive material which is different from the at least one second material (M2).

22. Device according to any one of the preceding claims, characterized in that the external porous layer is made of Pt, Au, Ir, TiN, Ir oxyde, Tungstene, ITO, semiconductors, alloys, conducting polymers or any other conducting material.

23. Device according to any one of the preceding claims, characterized in that the first electrode (10) is a first microelectrode and an array (1) of first microelectrodes (11) is provided, wherein the array (1) of first microelectrodes (11) is arranged in a determined configuration side–by–side, wherein each first microelectrode can be selected, the first microelectrodes (11) being insulated from each other, the conductors being insulated from each other, the zones (11) for local application to the living tissue being insulated from each other.

24. Device according to claim 23, characterized in that a plurality of third conductive zones (13) are located, respectively for a determined plurality of first microelectrodes (10), in the vicinity of the plurality of zones (11) of said determined first microelectrodes (11) of the array and are insulated from these zones (11) for application to the living tissue, said plurality of conductive third zones (13) belongs to a third conductive surface (3) supplemental to the first microelectrodes (11) and separate from the first microelectrodes (11), the third supplemental conductive surface (3) being used for all or part of the application against the living tissue, connection means being provided to ensure electric connection between said plurality of third conductive zones (13) of the supplemental third surface (3) for all said determined plurality of first microelectrodes (10) insulated from one another, so that the third conductive zones (31) of the third supplemental surface (3) are substantially equipotential, the supplemental third conductive surface (3) also being connected to at least one port (33) intended to be connected to an external return conductor (35) returning at least part of the signal and being formed to ensure focal stimulation from at least one of the determined plurality of first microelectrodes (10) and to act as common focusing means for several zones (11) of different determined first microelectrodes (10).

25. Device according to any one of the preceding claims, characterized in that the second mesoporous layer is fabricated by electrodeposition of the second conducting material through a template defining the pores, said template being composed of surfactant that is washed out after the electrodeposition.

26. Device according to claim 25, characterized in that the pores are made using micelles made of the surfactant and having a defined size and a defined concentration in the second conducting material, wherein the micelles are washed out leaving in the second material the pores of size and concentration corresponding to the defined size and defined concentration of the micelles.

27. Device according to claim 26, characterized in that the micelles are in such a concentration and such a uniform size so as to touch one another so as to leave at least some contiguous pores among said pores.

28. Device according to any one of the preceding claims, characterized in that for said mesoporous modification, a charge density of the second material lower than or equal 8 $C/cm^2$ is electrodeposited on the first geometric surface of the zone (11) for application.

29. Device according to claim 28, characterized in that the charge density of the second material has a value not exceeding 2 $C/cm^2$.

30. Device according to claim 28, characterized in that the charge density of the second material has a value not exceeding 1 $C/cm^2$.

31. Device to stimulate or record a signal to or from a living tissue, comprising at least one electrically conductive first electrode (10) and/or supplementary conductive surface (3) comprising a zone (11) for application to the living tissue and a conductor for sending or receiving a signal voltage to or from the zone (11), wherein the zone (11) for application to the living tissue is connected to said conductor,

characterized in that

the zone (11) for application to the living tissue belonging to the first electrode (10) and/or supplementary conductive surface (3) has a first geometric surface made of at least one first conducting material and modified by an external macroporous layer consisting in at least one second conducting material (M2por).

32. Device according to claim 31, characterized in that the macroporous layer has pore diameters within 50-1000 nm.

33. Device according to claim 31, characterized in that the macroporous layer has pore diameters of 500 nm.

FIG. 1A

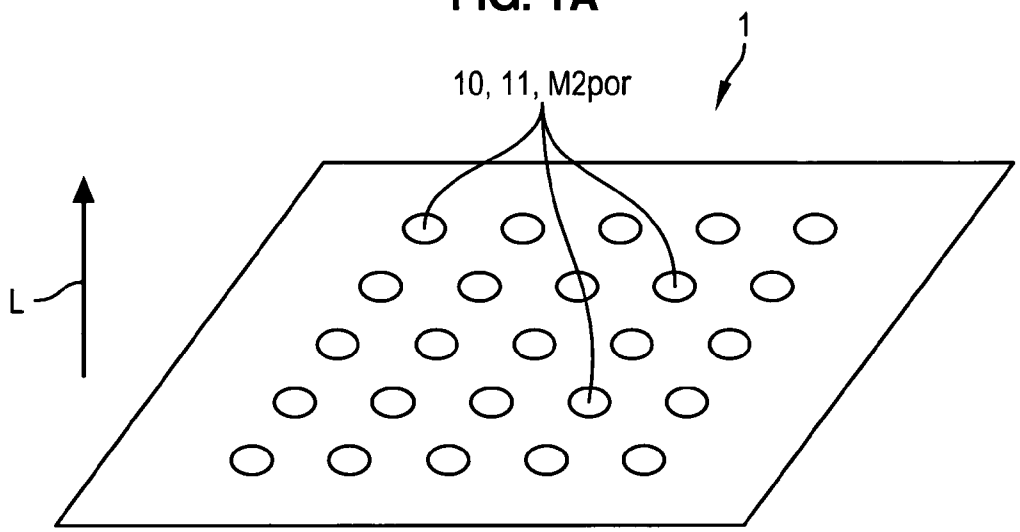



FIG. 1B

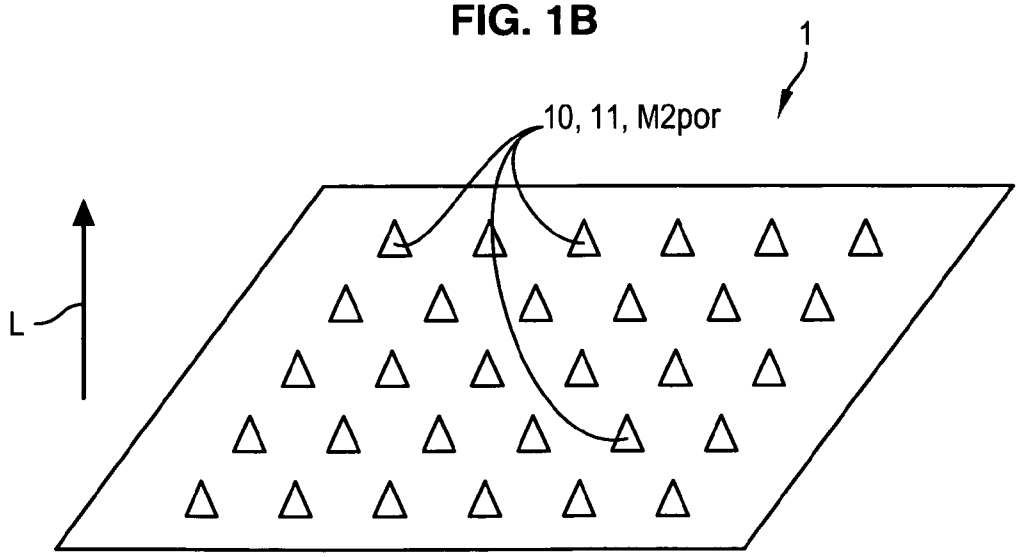


FIG. 1C

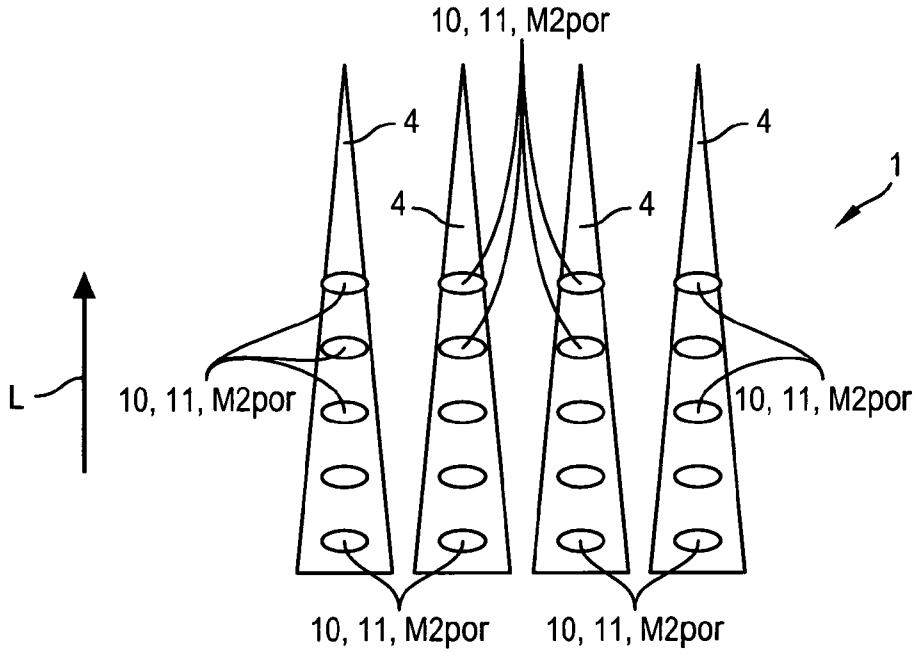

10, 11, M2por
4
4
4
4
1
L
10, 11, M2por
10, 11, M2por
10, 11, M2por
10, 11, M2por

FIG. 1D

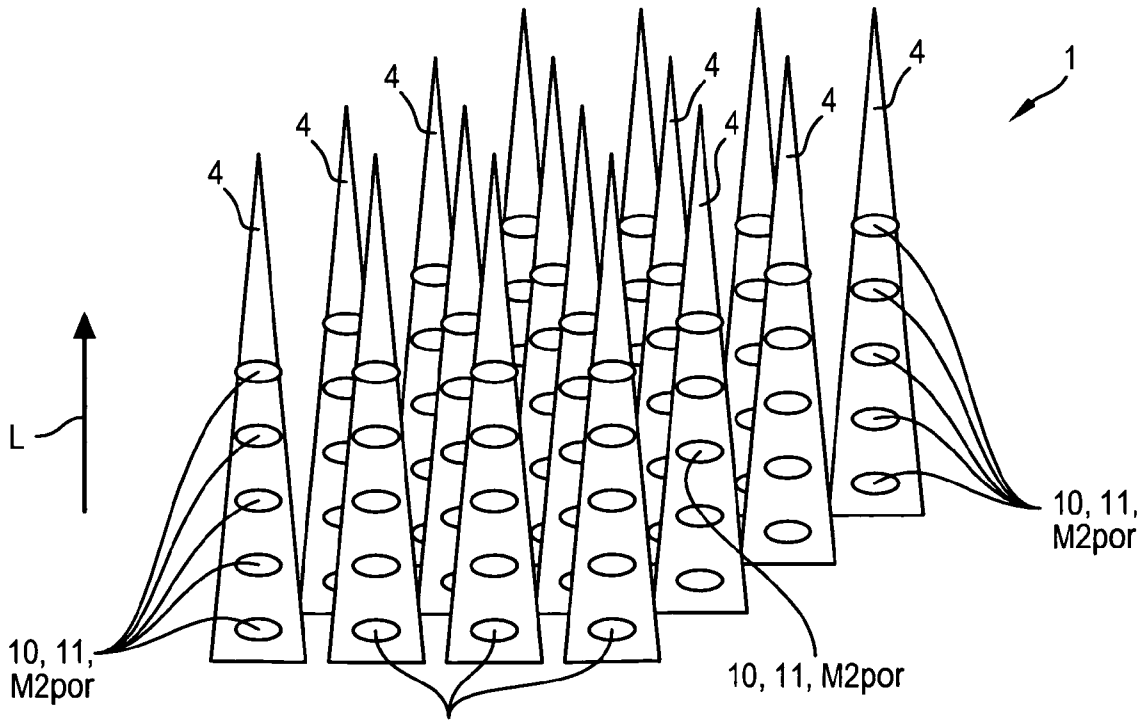

4
4
4
4
4
4
4
1
L
10, 11,
M2por
10, 11,
M2por
10, 11, M2por

FIG. 1E

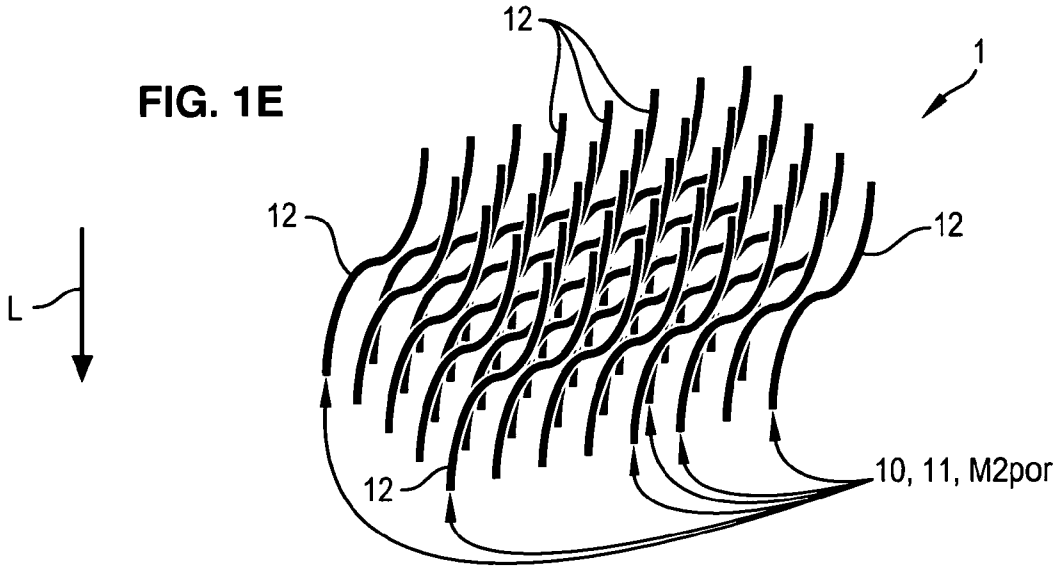



FIG. 1F

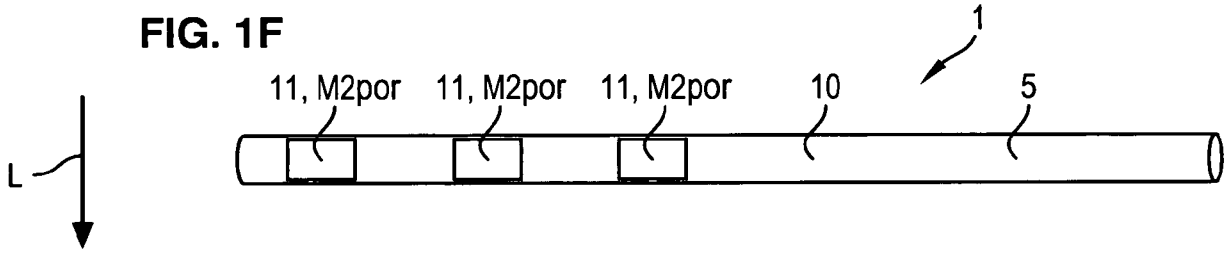



FIG. 1G

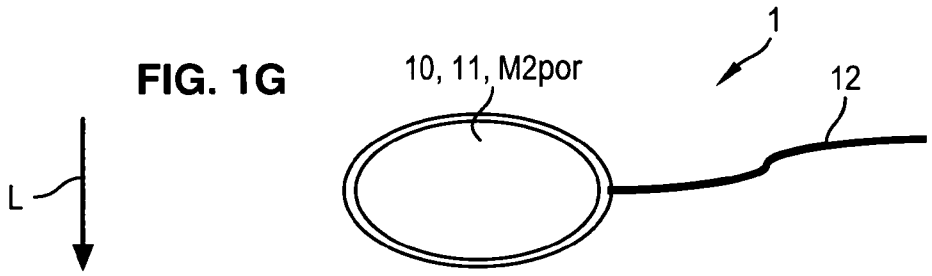



FIG. 1H

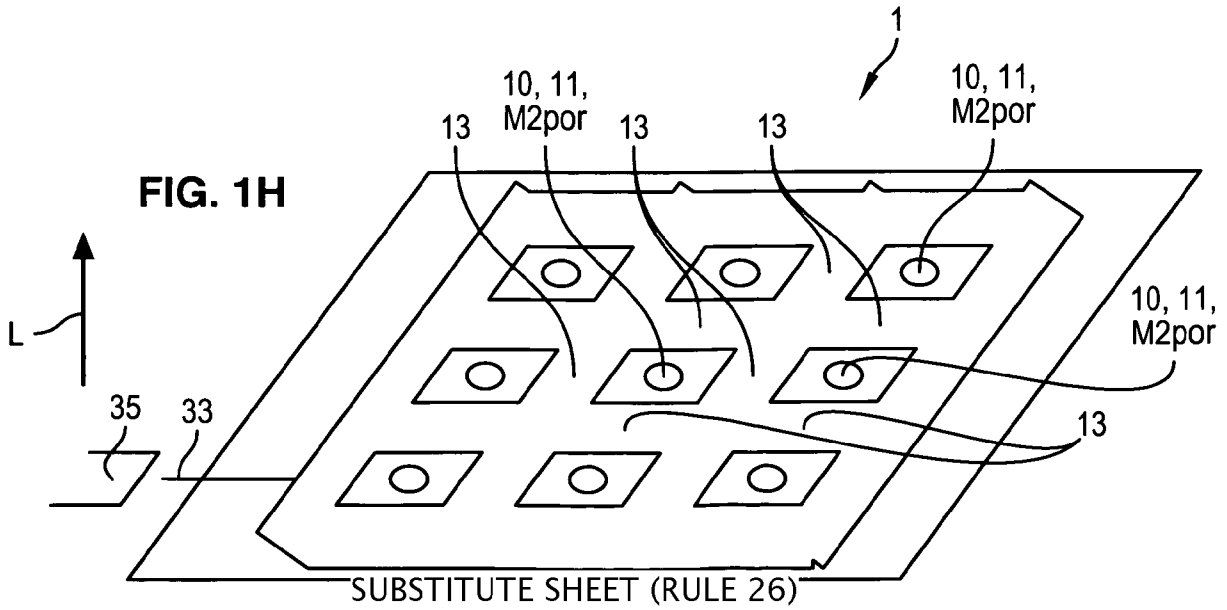


FIG. 2A

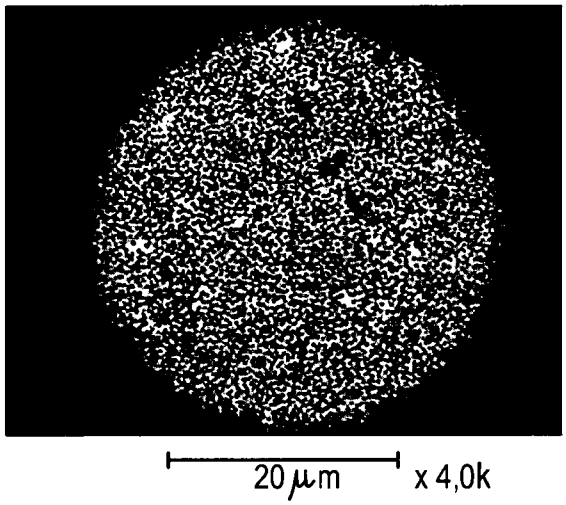



FIG. 2B

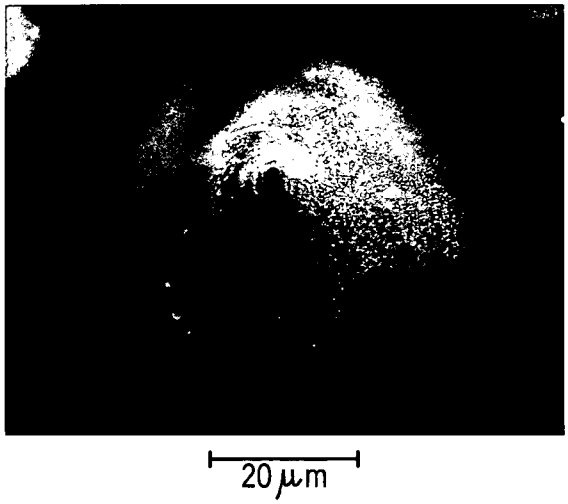



FIG. 3

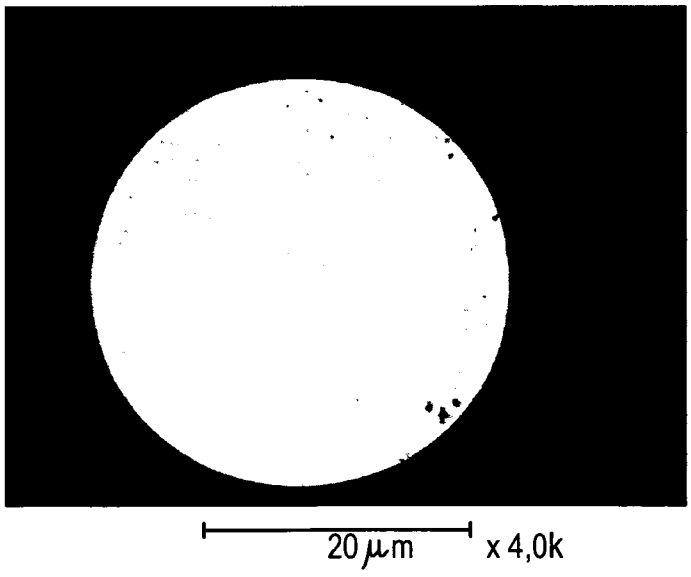


FIG. 4

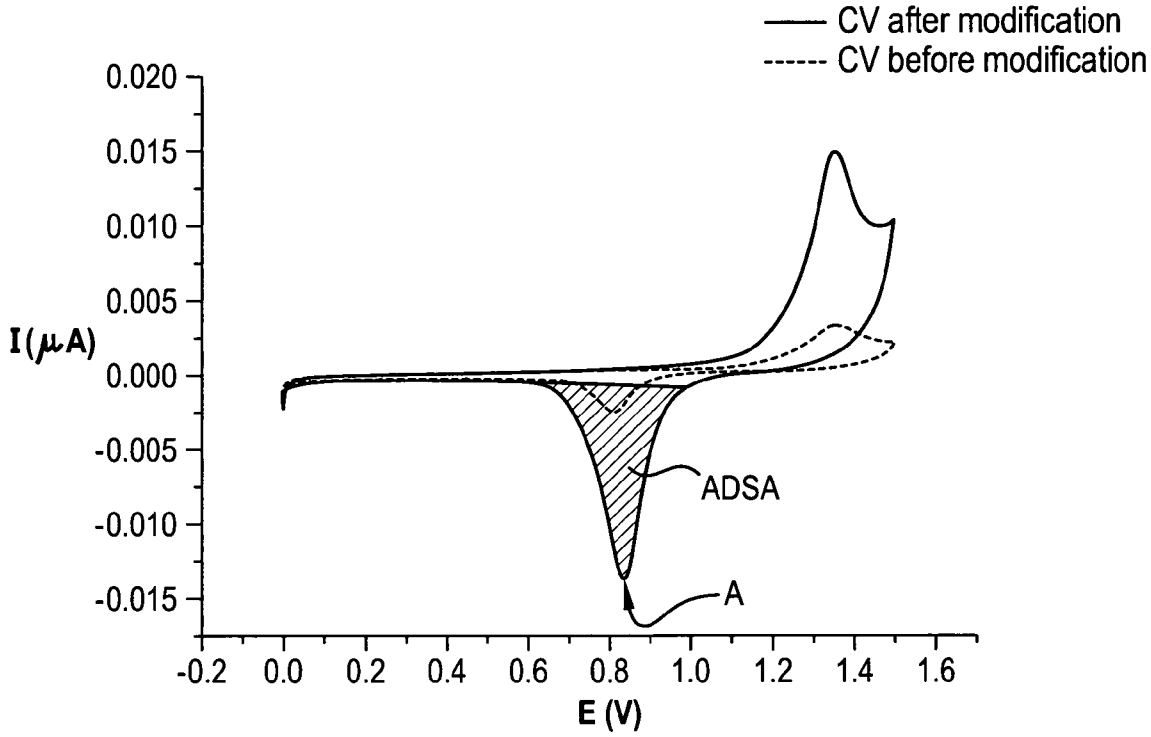

CV after modification
CV before modification
I(μA)
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
E (V)
ADSA
A

FIG. 5

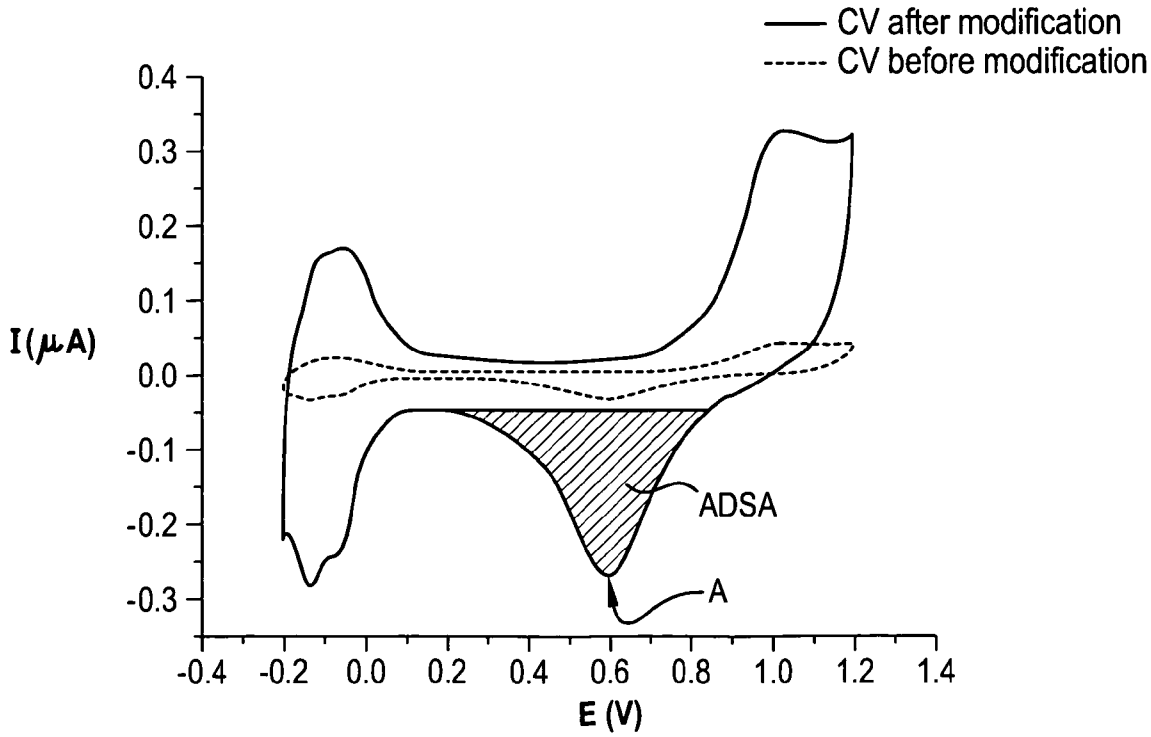

CV after modification
CV before modification
I(μA)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
E (V)
ADSA
A

FIG. 6A BEFORE MODIFICATION

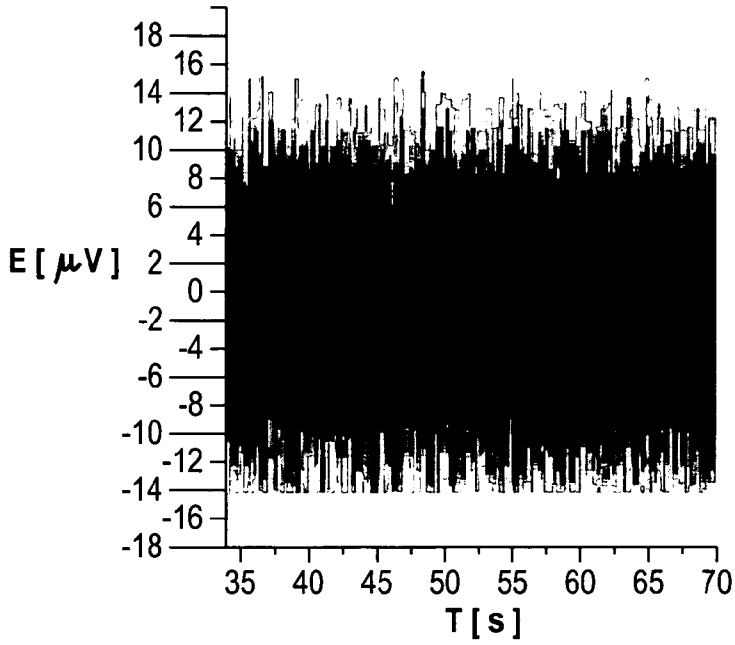



FIG. 6B AFTER MODIFICATION

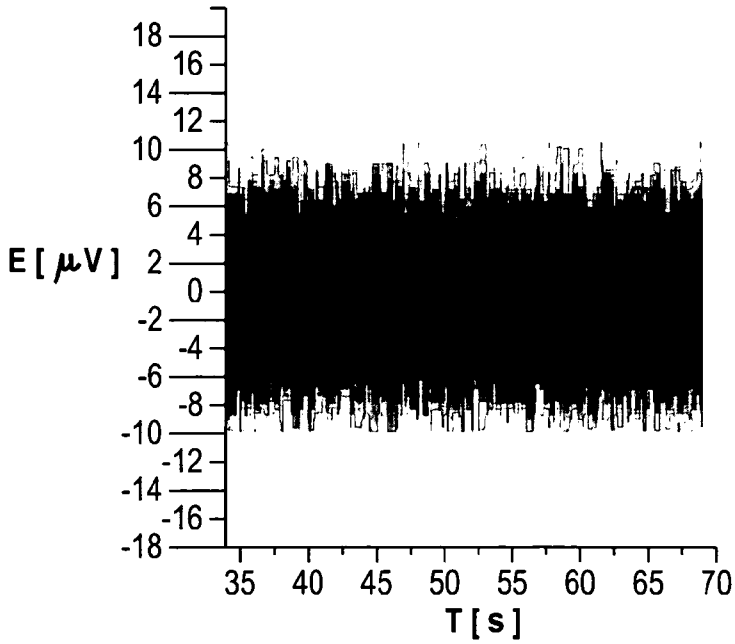


FIG. 7A

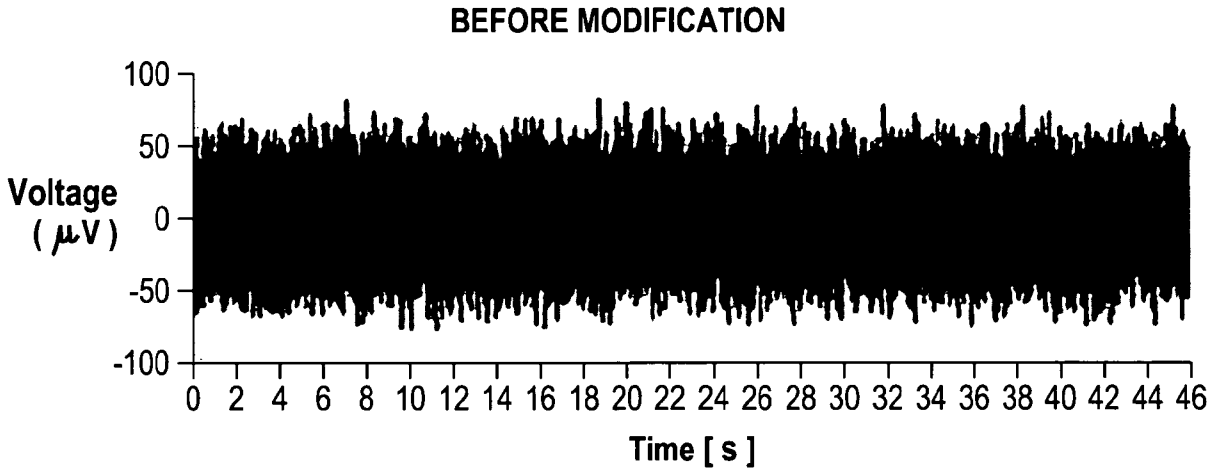
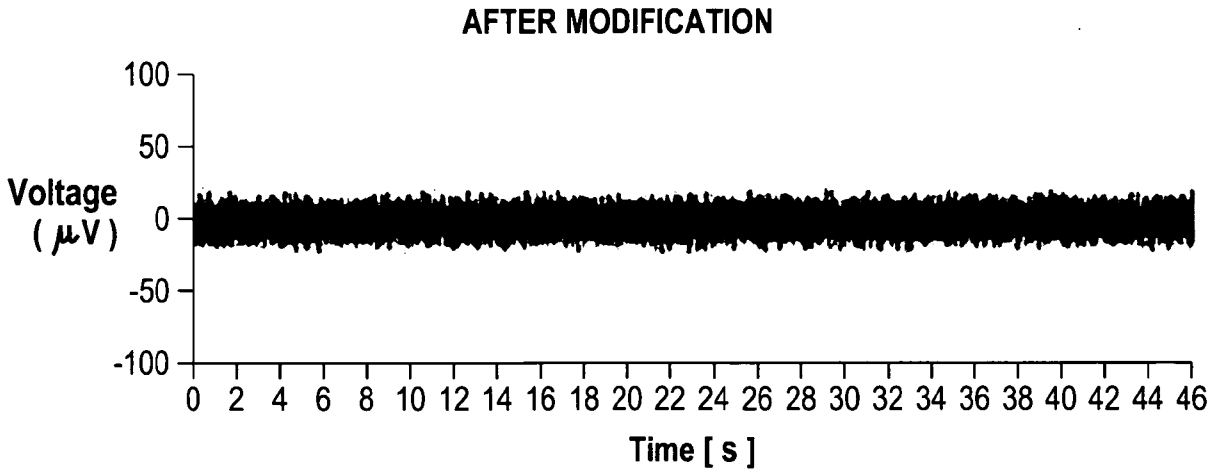

BEFORE MODIFICATION
100
50
0
-50
-100
Voltage
( μV )
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Time [ s ]

FIG. 7B


AFTER MODIFICATION
100
50
0
-50
-100
Voltage
( μV )
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Time [ s ]

# 8/21

FIG. 8

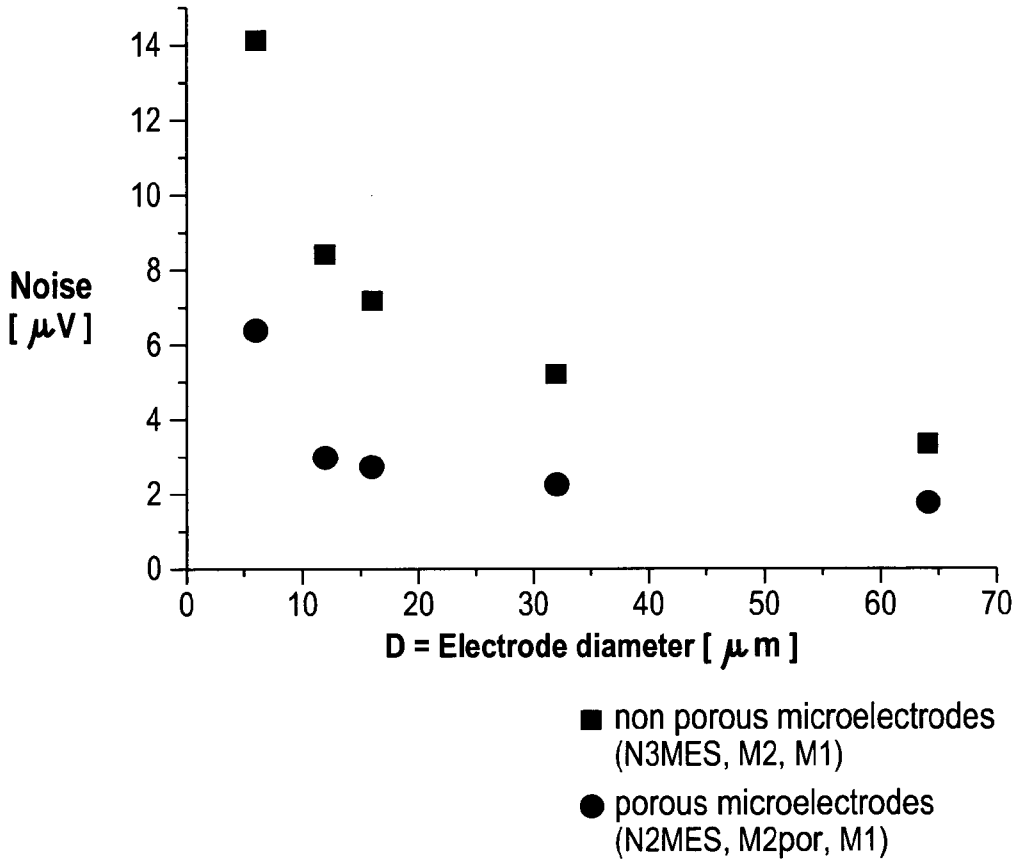



FIG. 9

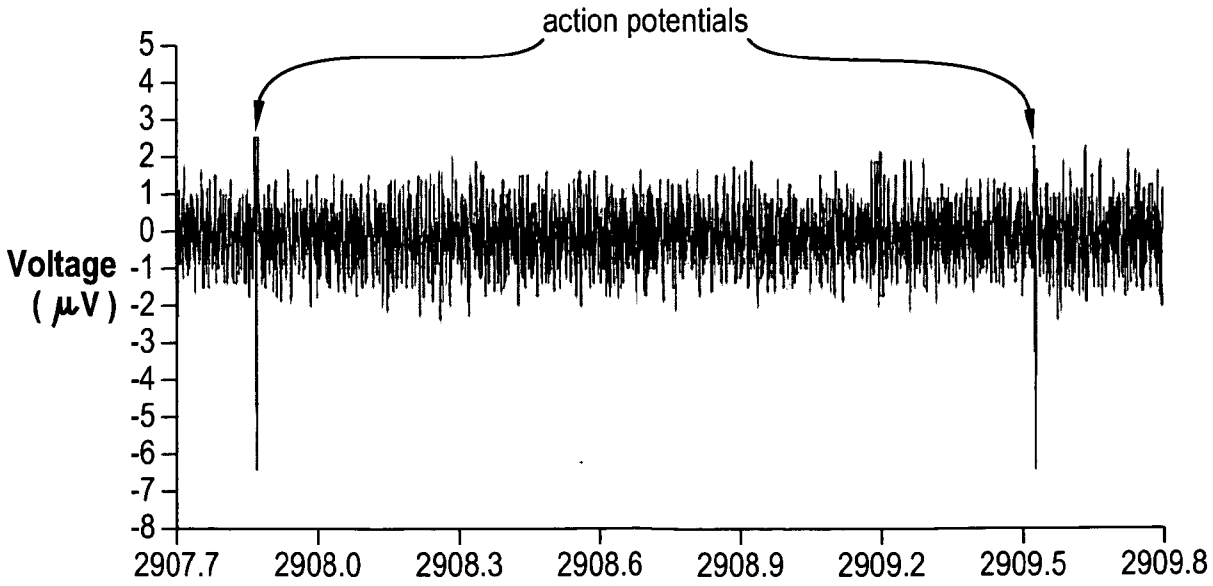


FIG. 10

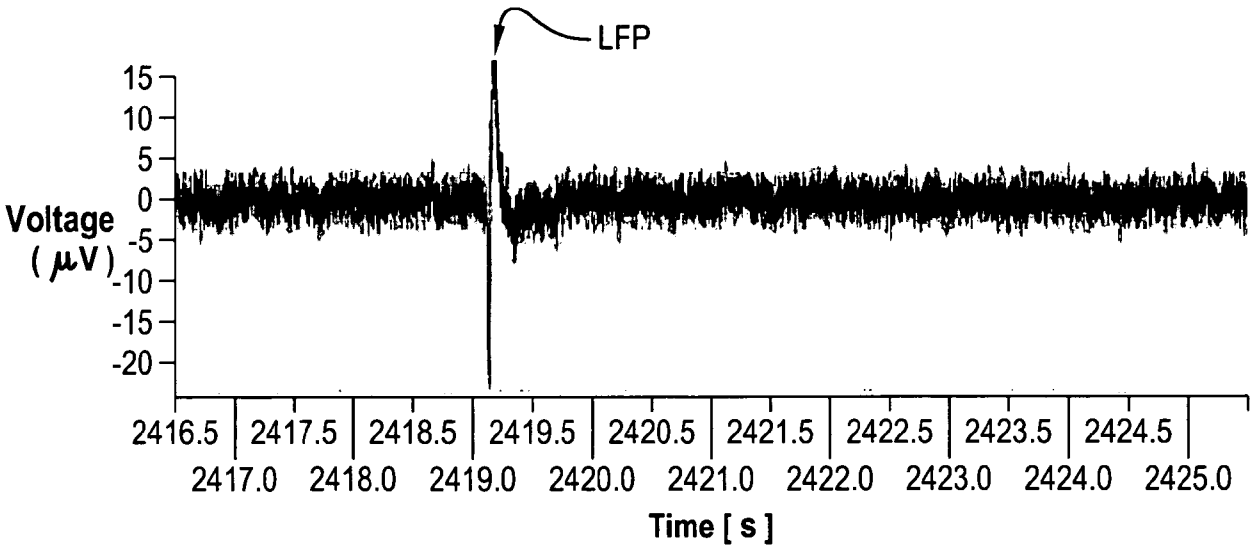

LFP
15
10
5
0
-5
-10
-15
-20
Voltage
( μV )
2416.5
2417.5
2418.5
2419.5
2420.5
2421.5
2422.5
2423.5
2424.5
2417.0
2418.0
2419.0
2420.0
2421.0
2422.0
2423.0
2424.0
2425.0
Time [ s ]

FIG. 11a

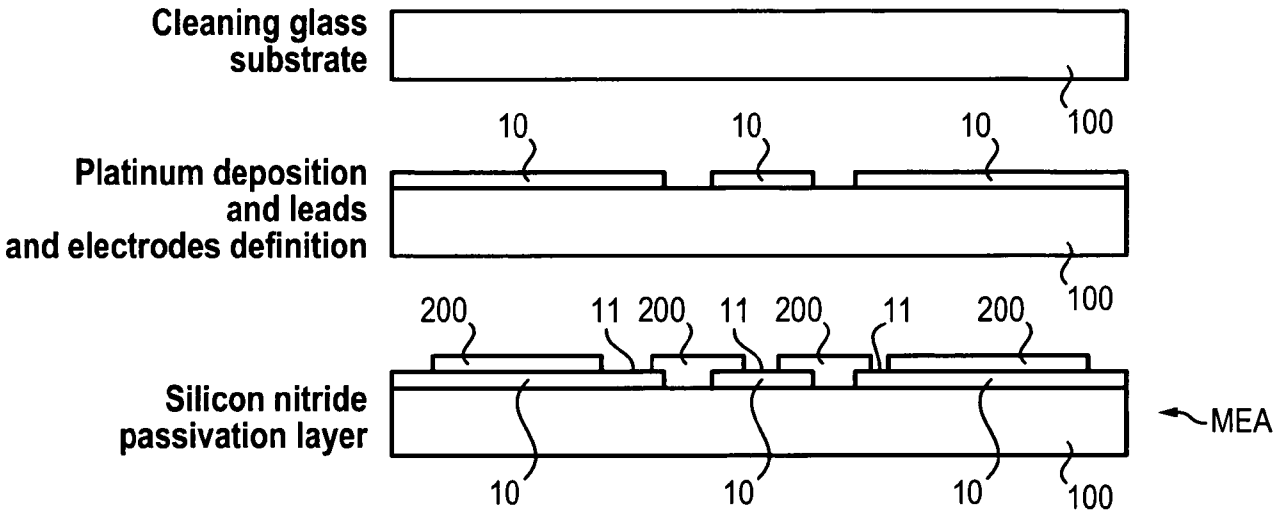



FIG. 11b

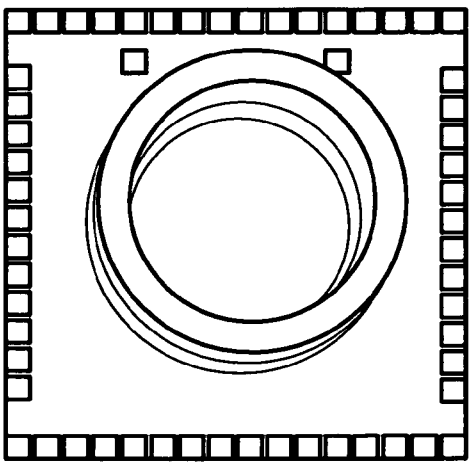


FIG. 11c

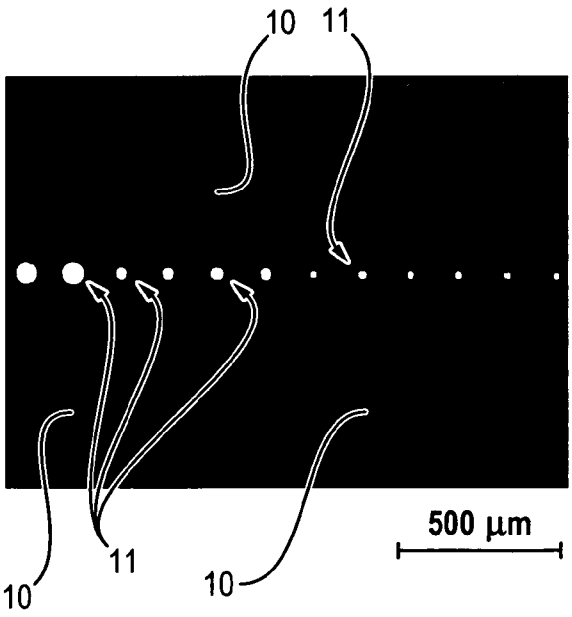


FIG. 11d

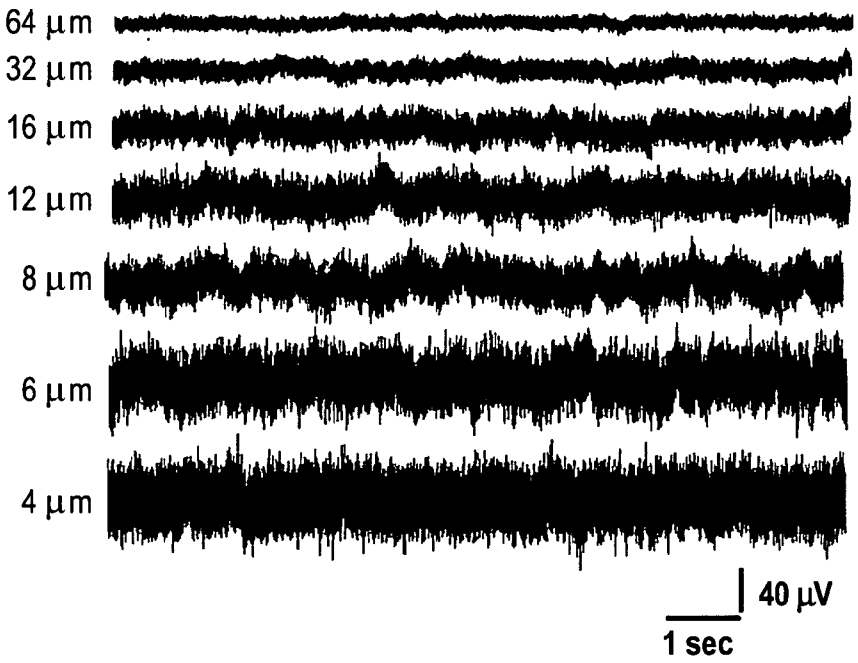



FIG. 11e

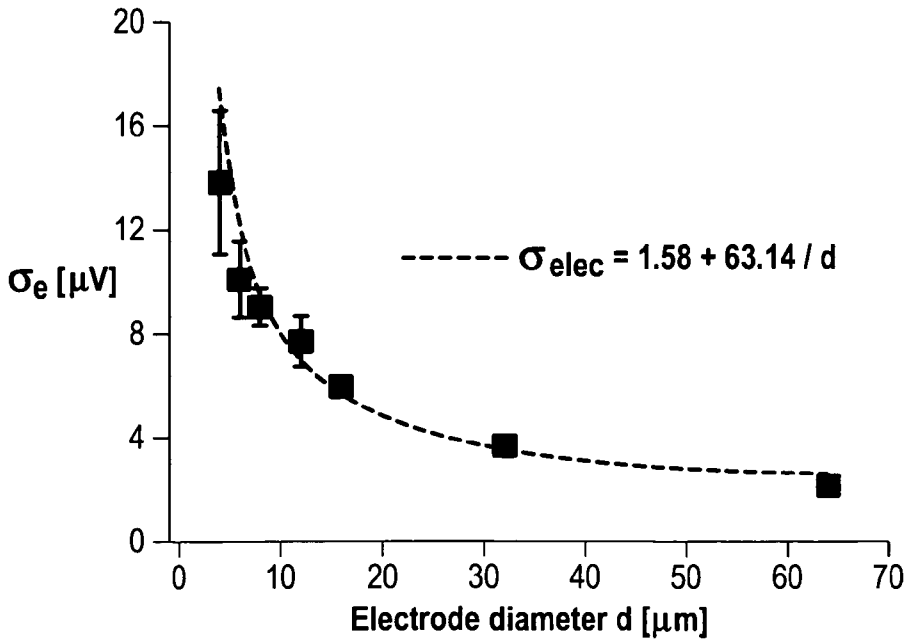


FIG. 11f

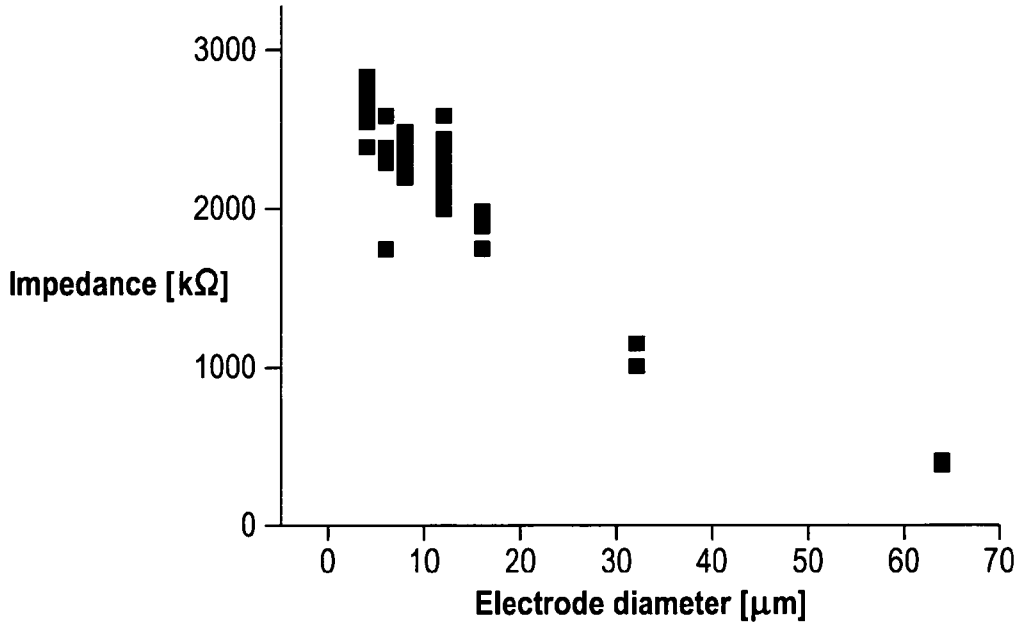



FIG. 11g

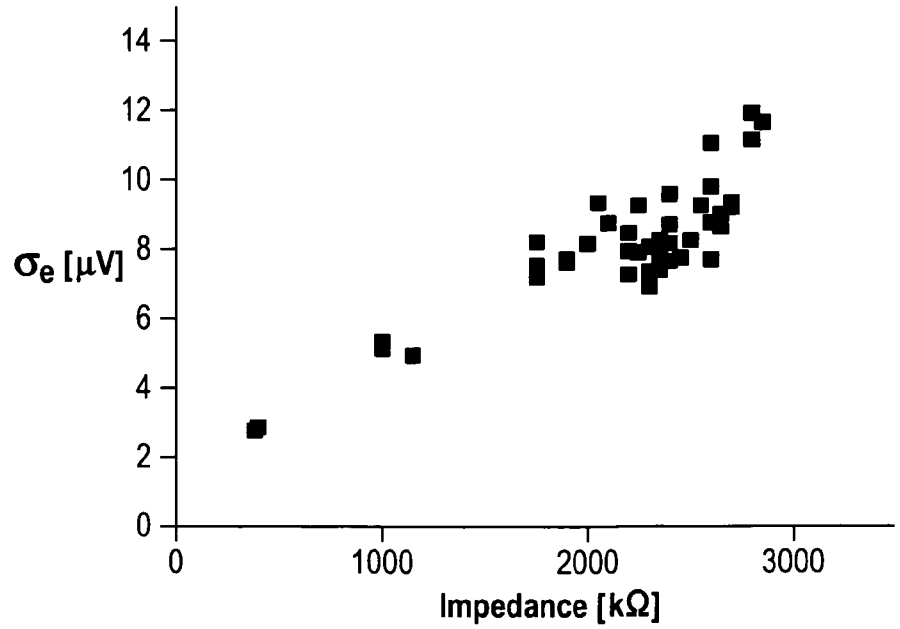


FIG. 12a

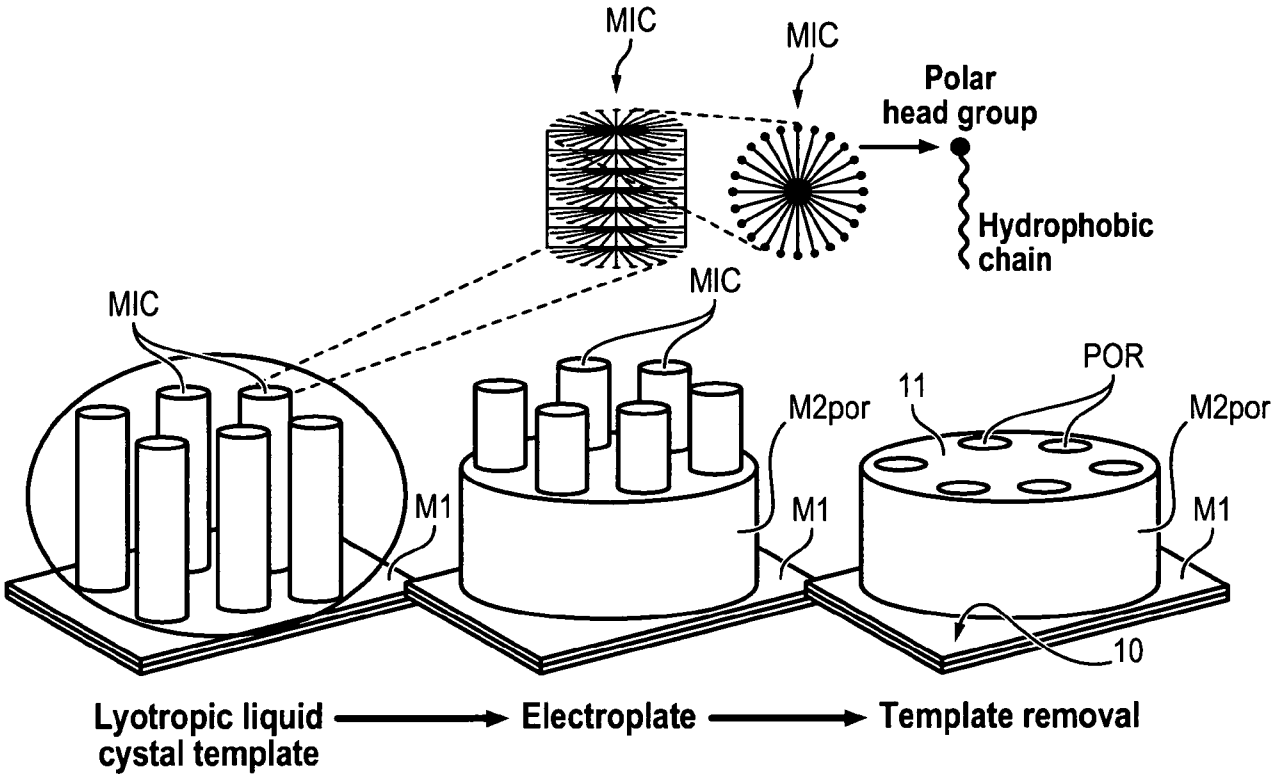



FIG. 12b

Before modification

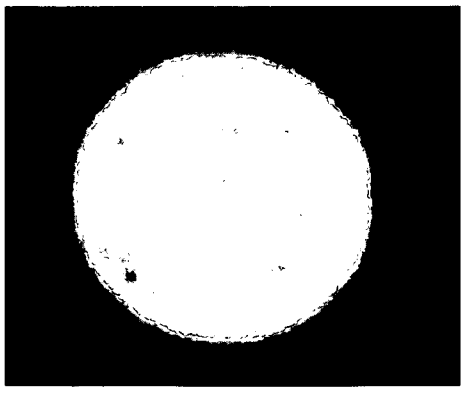



FIG. 12c

After modification

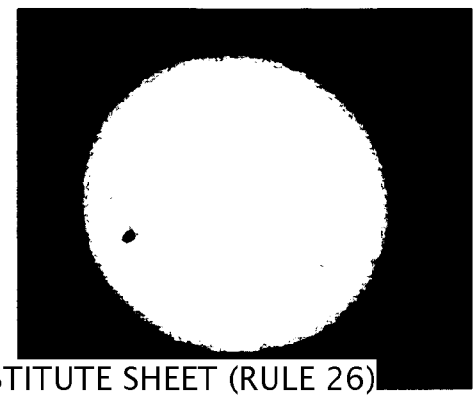


FIG. 13a

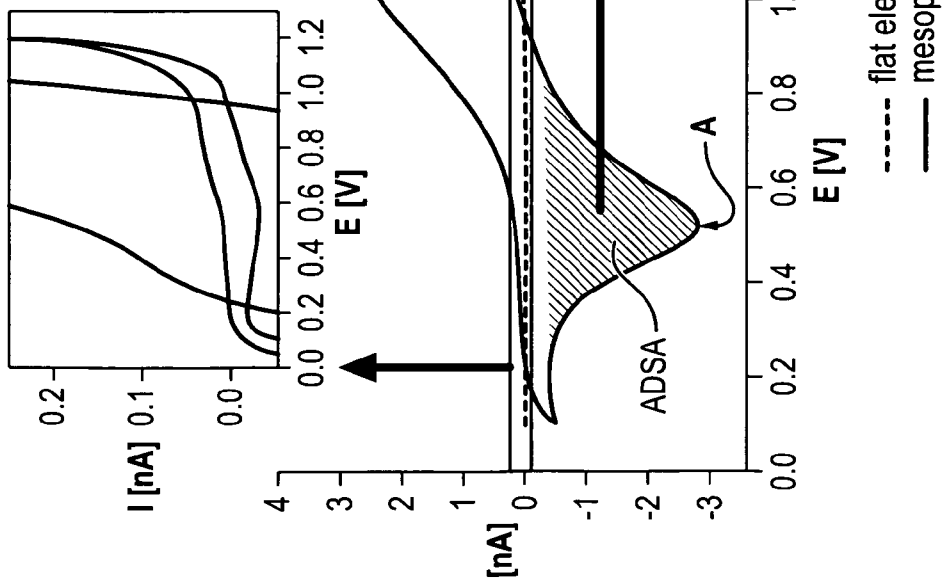



FIG. 13b

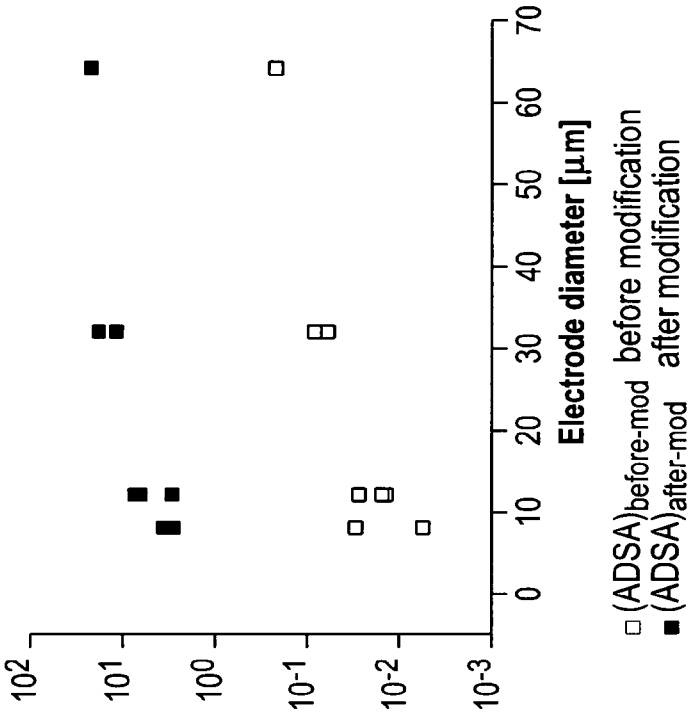
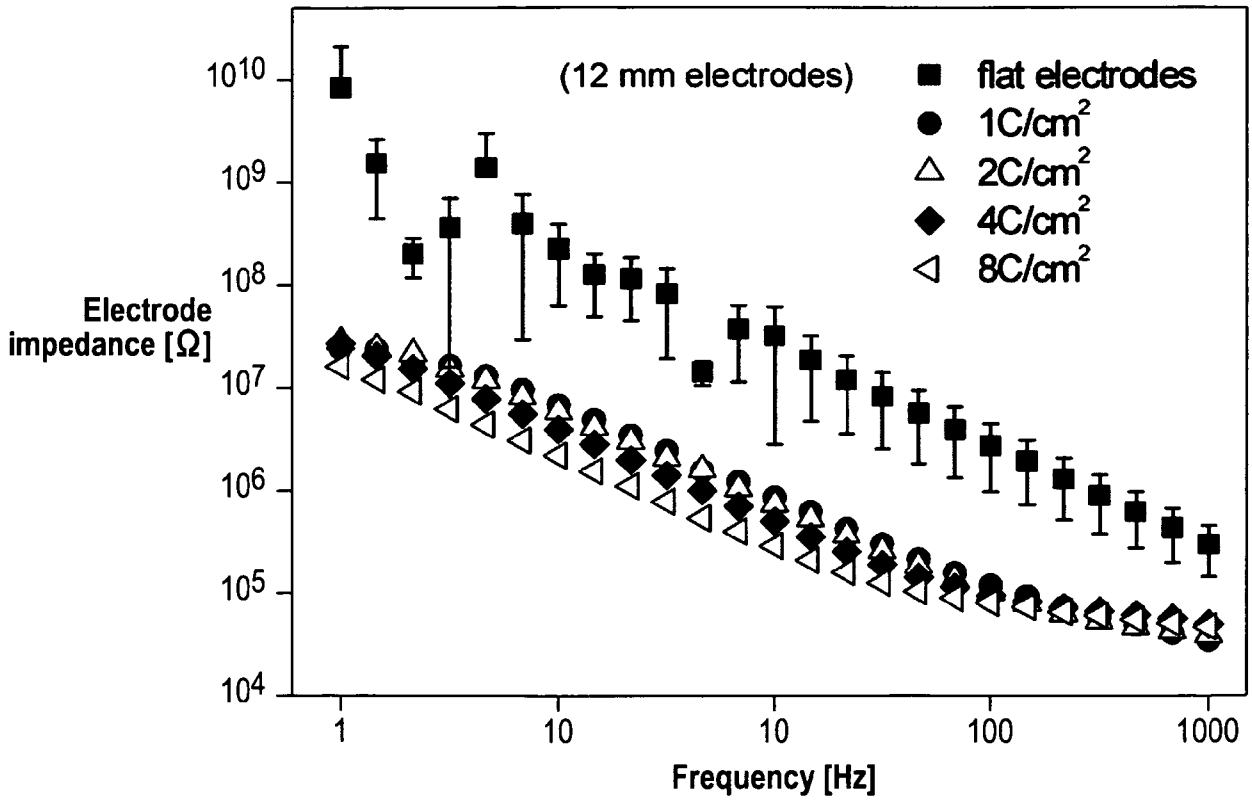


FIG. 14a


(12 mm electrodes)
flat electrodes
1C/cm²
2C/cm²
4C/cm²
8C/cm²
Electrode impedance [Ω]
10¹⁰
10⁹
10⁸
10⁷
10⁶
10⁵
10⁴
1
10
10
100
1000
Frequency [Hz]

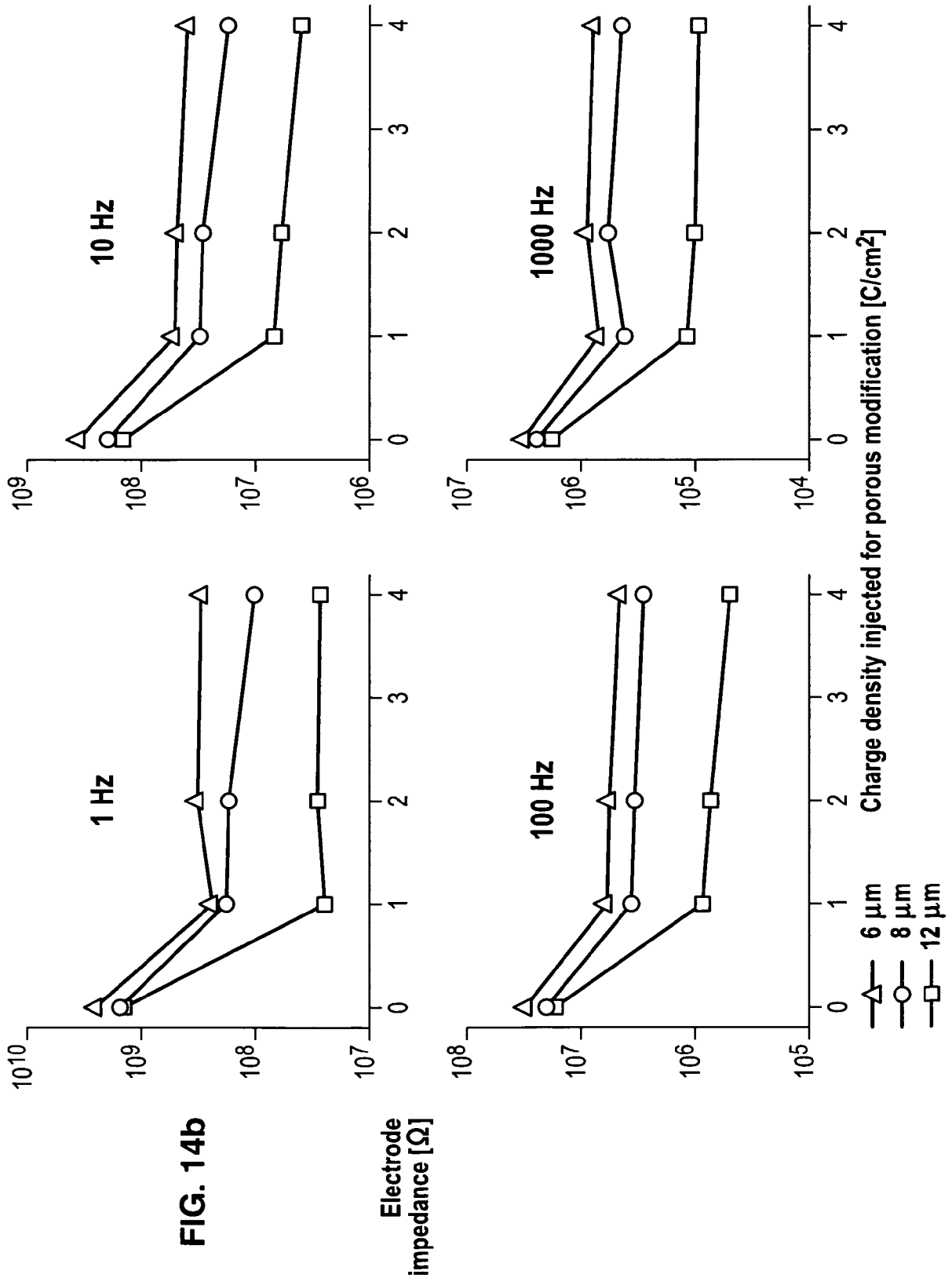

1 Hz
10 Hz
100 Hz
1000 Hz
Electrode impedance [Ω]
Charge density injected for porous modification [C/cm²]
6 µm
8 µm
12 µm

FIG. 14b

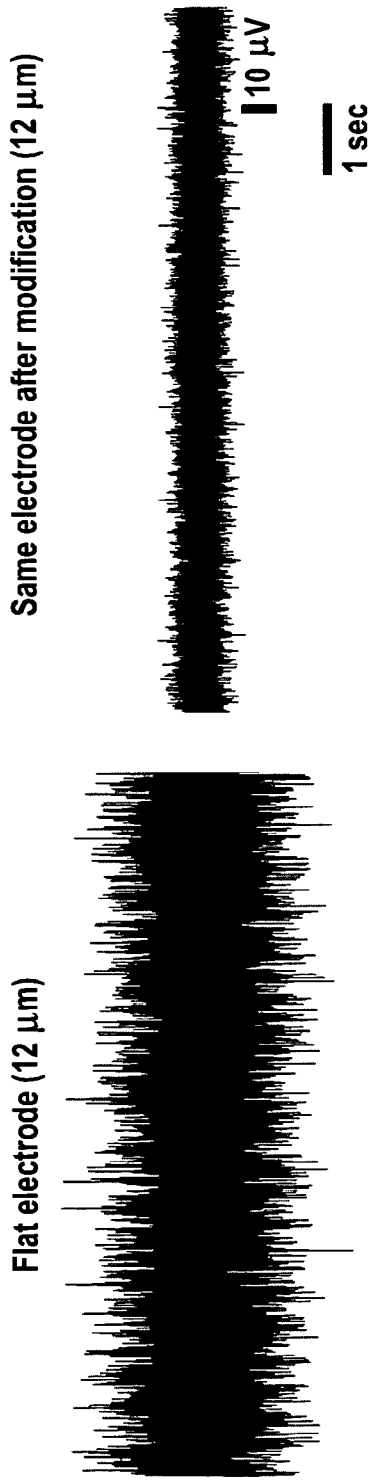

Same electrode after modification (12 µm)
10 µV
1 sec
Flat electrode (12 µm)

FIG. 15a

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FIG. 15b

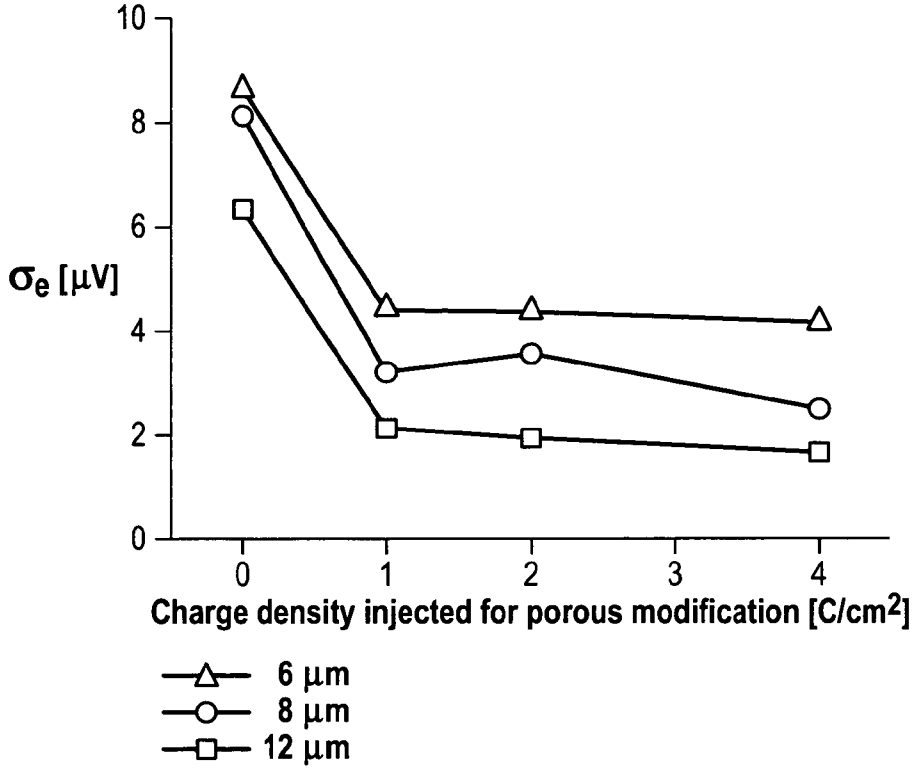


FIG. 15c

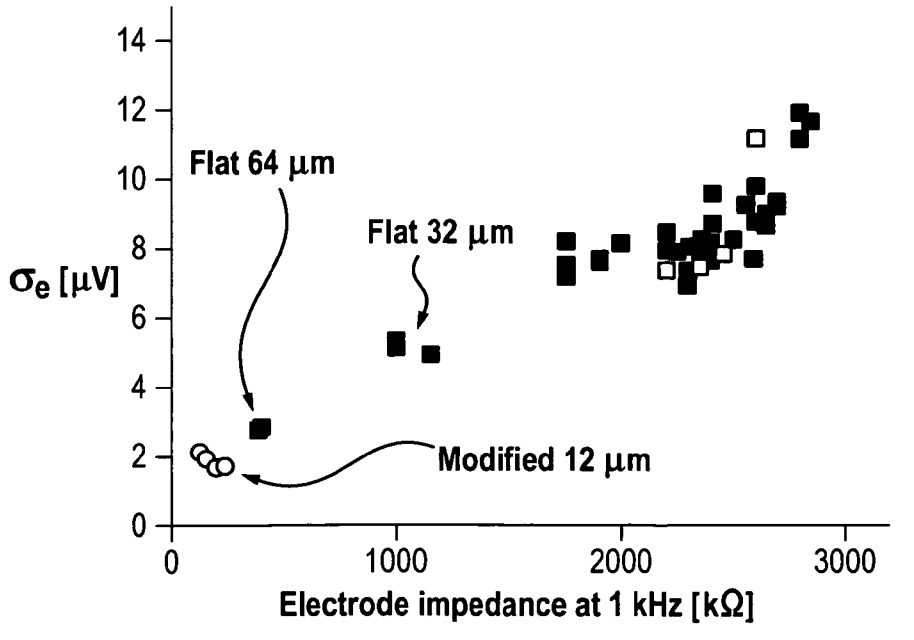

FIG. 15d

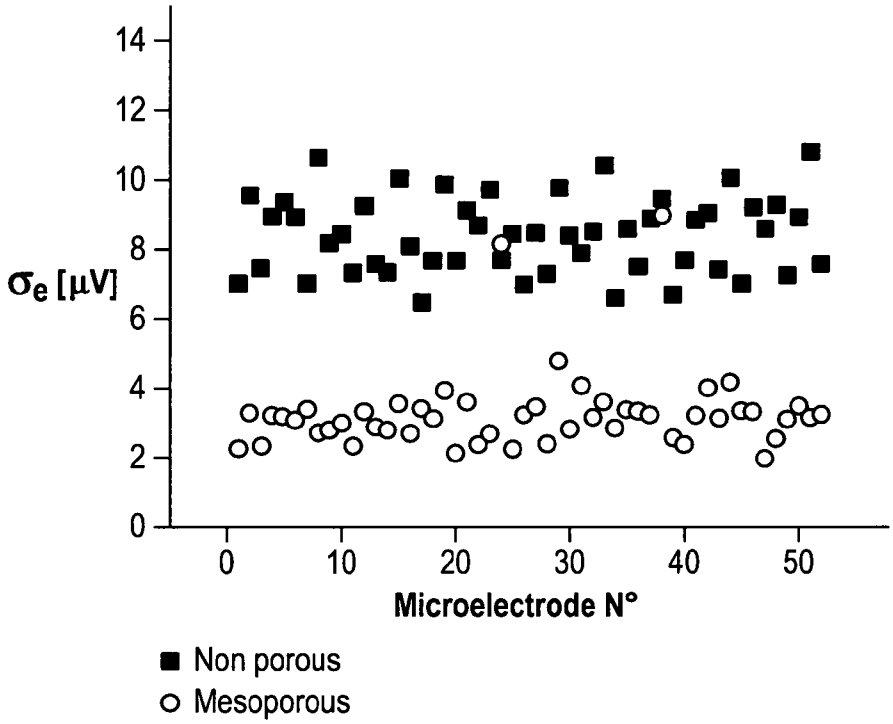


FIG. 15e

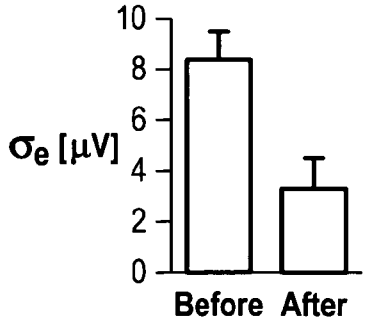


FIG. 15f

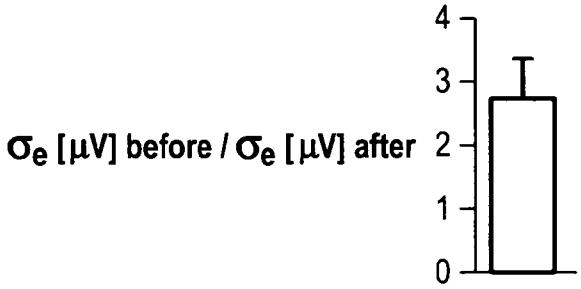

FIG. 16a

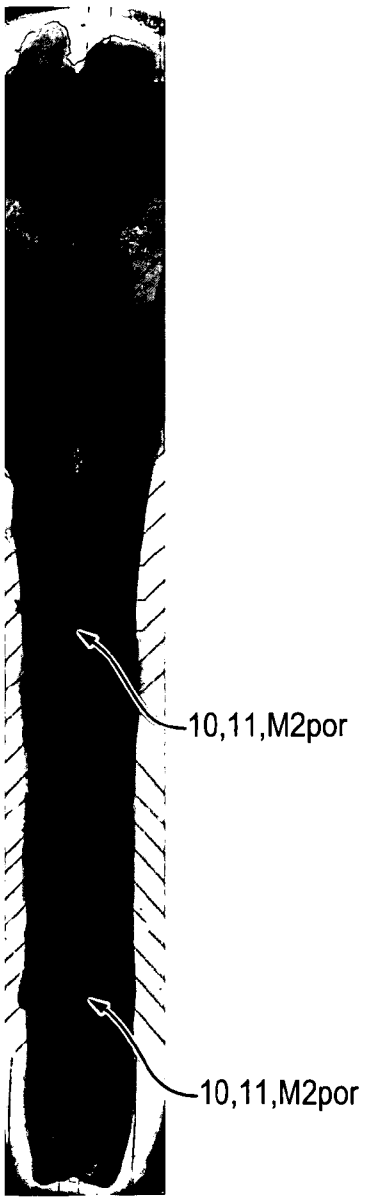


FIG. 16b

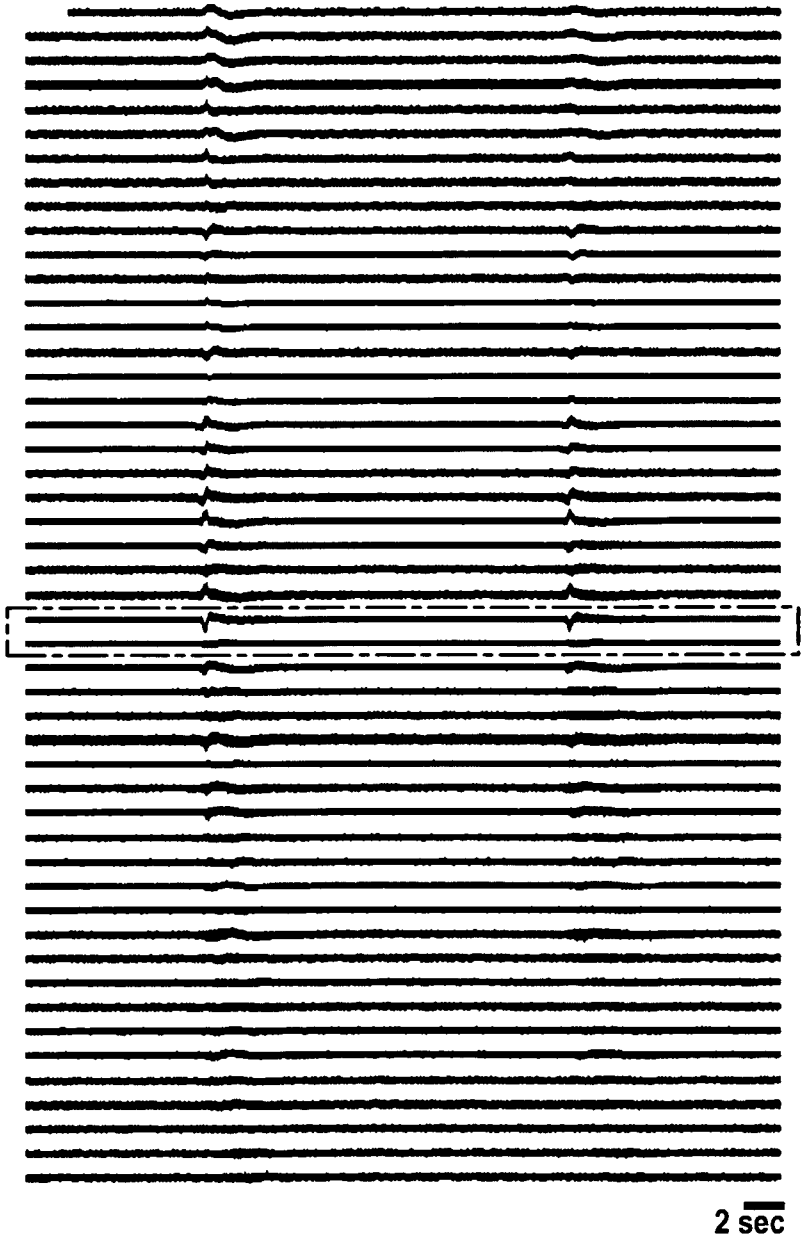

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FIG. 16c

Raw data

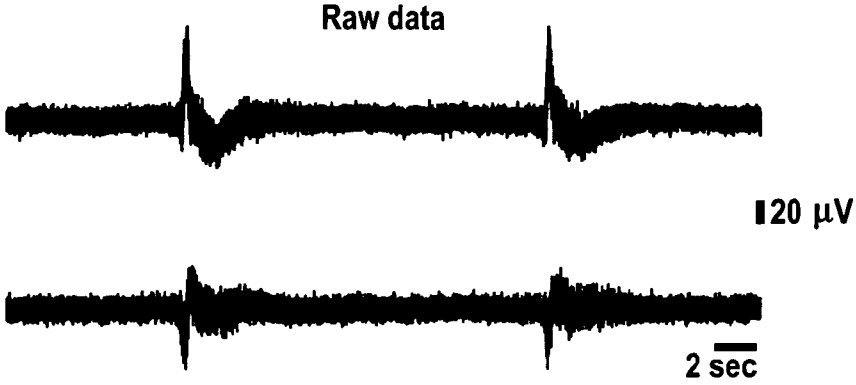


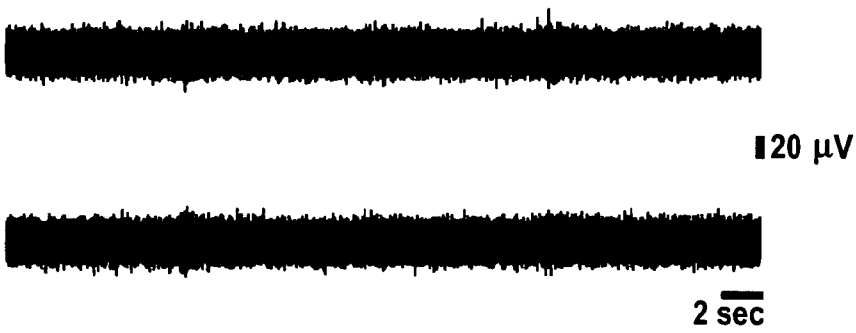

FIG. 16d

High-pass filtered data




FIG. 16e

High-pass filtered data surimposed on flat electrode noise



# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/059431

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N1/36 A61N1/05
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>Y | US 2009/248113 A1 (NIMER EMAD [IL] ET AL)<br>1 October 2009 (2009-10-01)<br>abstract; figures 1a, 2a<br>paragraphs [0001], [0022] - [0024],<br>[0111], [0113], [0118] - [0122],<br>[0126], [0135], [0145] - [0146]<br>----- | 1-23,<br>25-33<br>24 |
| Y | WO 2009/053333 A1 (CENTRE NAT RECH SCIENT<br>[FR]; GROUPE ECOLE SUPERIEURE D INGE [FR];<br>UNIV) 30 April 2009 (2009-04-30)<br>cited in the application<br>abstract; figure 3<br>page 1, lines 1-28<br>page 3, lines 17-34<br>page 14, lines 26-36<br>----- | 24 |

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☒ See patent family annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 August 2011 | 22/08/2011 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Pfeiffer, Uwe |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/059431

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | WO 2006131912 A2 | 14-12-2006 |
| WO 2009053333 A1 | 30-04-2009 | CA 2702277 A1 | 30-04-2009 |
| | | EP 2217322 A1 | 18-08-2010 |
| | | FR 2922460 A1 | 24-04-2009 |
| | | JP 2011500240 A | 06-01-2011 |